Department of Environment and Agriculture

Regulation of Health-Promoting Compounds in Ripe Mango Fruit

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: M.D.K Vithana

Date: 29/11/2017

Dedication

To: my ever loving husband Dushsantha and beloved children Sandith and Amadi for their endless love and unconditional support.

I'm blessed to have you in my life!!

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Abstract

Mango (*Mangifera indica* L.) is one of the choicest fruit in the world. The interest in its level of nutraceuticals is increasing along with the evidence of their ability to reduce the risk of several chronic ailments. Thus, the aim of my PhD study was to investigate different pre- and post-harvest factors and conditions that may increase the level of health-promoting compounds in ripe mango fruit. To accomplish this aim, the effects of pre-harvest spray application of different concentrations of $FeSO_4$ (Fe^{2+}), $MgSO_4$ (Mg^{2+}) and $MnSO_4$ (Mn^{2+}), harvest maturity (hard green, sprung stage, half ripe and tree ripe), postharvest fruit ripening, chilling (5 °C) over standard non-chilling (13 °C) low temperature storage for different durations (12 d or 24 d) and different concentrations of postharvest elicitors methyl jasmonate (MeJA), salicylic acid (SA) and nitric oxide (NO) on the concentrations of health-promoting compounds in the pulp and peel of the ripe mango fruit were investigated.

$FeSO_4$, $MgSO_4$ and $MnSO_4$ treatments increased the concentration of lupeol in the peel irrespective of the concentration compared to the control. Aqueous solutions of all above nutrients 0.3% and 0.2% significantly increased the level of total carotenoids and mangiferin in the pulp respectively. The concentrations of gallic and ferulic in the peel and chlorogenic acid in the pulp were the highest with the pre-harvest spray application of 0.2% $FeSO_4$ solution.

The fruit harvested at sprung stage exhibited in highest concentrations of lupeol, mangiferin, vanillic acid, ferulic acid and caffeic acid in both pulp and peel and gallic acid, chlorogenic acid and total phenols in the peel of ripe fruit. The highest concentrations of ascorbic acid and total carotenoids in pulp and total antioxidant capacity in peel were recorded in the fruit harvested at tree ripe stage. Whilst, the fruit harvested at half ripe stage exhibited the highest antioxidant capacity in the ripe pulp. A significant increase in the concentrations of lupeol, mangiferin, phenolic acids, total antioxidants and total carotenoids was observed in the pulp and/or peel of the fruit of 'Kensington Pride' and 'R2E2' during the post-climacteric ripening phase.

The storage at 5 °C (chilling temperature) significantly increased the concentrations of lupeol in pulp and peel and chlorogenic and caffeic acids in the pulp of ripe mango fruit compared to 13 °C. The ripe mango fruit previously stored

at standard cold storage temperature (13 °C) offered the highest concentrations of mangiferin, gallic, chlorogenic, vanillic, ferulic, and caffeic acids, total phenols, antioxidants and carotenoids in the peel compared to fruit stored at 5 °C. Extended storage (24 d) significantly reduced the concentrations of lupeol and chlorogenic acid in pulp and peel and gallic acid in the pulp compared to storage for 12 d. However, 24 d cold storage significantly increased the concentrations of vanillic acid, total phenols, total antioxidants and ascorbic acid in the pulp and caffeic acid in both pulp and peel compared to 12 d storage. Chilling injury symptoms were developed in the ripe fruit that were stored at 5 °C for 24 d.

Significantly higher concentrations of mangiferin, gallic and chlorogenic acids, total phenols and antioxidants in both pulp and peel, total carotenoids and ascorbic acid in the pulp and lupeol and caffeic acid in the peel of ripe mango fruit after the fumigation with MeJA for 24 h (10^{-5} M and/or 10^{-4} M MeJA) at green mature stage compared to untreated control. The concentrations of chlorogenic and ferulic acids in both the pulp and peel and lupeol, mangiferin, vanillic acid, total carotenoids and ascorbic acid in the pulp of ripe mango fruit were significantly higher with the dip treatment in SA solutions (2 mmol L^{-1} and/or 3 mmol L^{-1}) for 10 min at green mature stage compared to the untreated control. The total phenol concentration in the peel and the concentrations of gallic acid in both the pulp and peel were significantly lower after the SA treatment. Fumigation with NO for 2 h (20 and/or 40 μL L^{-1}), at green mature stage significantly increased the concentrations of lupeol, mangiferin, gallic and chlorogenic acids in the pulp and peel, vanillic, ferulic and caffeic acids, total phenols, total antioxidants, total carotenoids and ascorbic acid in the pulp of ripe mango fruit.

Thus, pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.2% and/or 0.3%), harvesting fruit at sprung stage and fruit at post-climacteric ripening phase were identified for the first time as promising ways to obtain ripe mango fruit with higher levels of lupeol, mangiferin, phenolic acids and other health-promoting compounds. Similarly, storage of green mature fruit at 5 °C for 12 d prior to ripening and postharvest MeJA, SA and NO treatments were also identified for the first time as potential ways to obtain ripe mango fruit with higher levels of above health-promoting compounds.

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List of symbols and abbreviations

×	Multiply / interaction
≤	Less than or equal
>	More than
±	Plus or minus
/	Divide
/	Or
=	Equal to
´	Minute(s)
%	Per cent
°	Degree
°C	Degree celcius
$	Dollar
&	And
β	Beta
µg	Microgram(s)
µL	Microlitre(s)
ANOVA	Analysis of variance
C	Carbon
CI	Chilling injury
cm	Centimetre(s)
cm^3	Cubic centimetre(s)
Co.	Company
CO_2	Carbon dioxide
CRD	Completely randomized design
d	Day(s)
DAD	Diode array detector
DNA	Deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
DW	Dry weight
E	East
EDTA	Ethylenediaminetetraacetic acid

et al.	et alia
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization statistical database
Fe^{2+}	Ferrous ion
$FeSO_4$	Ferrous sulphate
FID	Flame ionization detector
Fig.	Figure
FW	Fresh weight
g	Gram(s)
GAE	Gallic acid equivalent
h	hour
ha	Hectares
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
JA	Jasmonic acid
kg	Kilogram(s)
L	Litre(s)
LSD	Least significant difference
Ltd.	Limited
M	Molar
mAU	Miliabsorbance units
mB	Milibar(s)
MeJA	Methyl jasmonate
mg	Miligram(s)
Mg^{2+}	Magnesium ion
$MgSO_4$	Magnesium sulphate
min.	Minute
mL	Mililitre(s)
mM	Milimolar
Mn^{2+}	Manganese ion
$MnSO_4$	Manganese sulphate
mmol	Milimole(s)
mol	Mole(s)

Mt	Metric ton(s)
N	Newton(s)
N_2	Nitrogen
nm	Nanometre(s)
NO	Nitric oxide
NaF	Sodium floride
NS	Non-significant
NSW	New South Wales
NT	Northern Territory
P	Probability
PAL	Phenylalanine ammonia lyase
pmol	Pekomole(s)
QLD	Queensland
R^2	Coefficient of determination
RDI	Regulated deficit irrigation
S	South
s	Seconds
SA	Salicylic acid
SD	Standard deviation
TEAC	Trolox equivalent antioxidant capacity
USDA	United States Department of agriculture
UV/VIS	Ultra violet/ visible spectrum
WA	Western Australia
viz.	Namely
v/v	Volume by volume
w/v	Weight by volume

CHAPTER 1

General introduction

Mango fruit (*Mangifera indica* L.) is a rich source of phytochemicals such as lupeol, mangiferin, phenolic acids, ascorbic acid and carotenoids with significant health benefits (Godoy and Rodriguez-Amaya, 1994; Ma et al., 2011; Masibo and He, 2008; Masibo and He, 2009; Saleem, 2009; Srivastava et al., 2015). Lupeol is a bioactive compound which is renowned for its capability of selective control of cancerous cells as well as for its capability in reducing the risk of several other degenerative diseases (Saleem, 2009), mangiferin, which is categorized under the flavanoid group has an ability in reducing the risk of heart diseases and cancer mainly due to its free radical scavenging and iron-complexion abilities (Puccio and Kœnig, 2002). Mango fruit has a significant antioxidant capacity which is mainly contributed by gallic, chlorogenic and vanillic acids (Kim et al., 2007; Palafox – Carlos et al., 2012b).

Cardiovascular diseases and cancer are the leading causes of death and the prime economic burdens worldwide (WHO, 2017a; WHO 2017b). Nonetheless, both these diseases are preventable to a considerable degree as over 30% of cancers and about 50% of the cardiovascular disease incidences are related to dietary and behavioural risks (WHO, 2017a and WHO, 2017b). Proper nutrition could possibly play a protective role in reducing the risk of these diseases and also would provide a feasible mean to reduce the economic encumberance caused by the costs of treatments (Kruger et al., 2014).

Mango is the fifth most produced fruit crop globally, which is grown over an area of about 5.4 million ha in about 100 countries in the world with an annual production over 42 million Mt (FAOSTAT, 2017). According to FAOSTAT (2017), the world mango production is increasing steadily over the years with an annual growth rate of mango trade about 3.58%. Thus, value addition to this fruit of choice by increasing its concentration of health-promoting compounds would possibly provide a noteworthy contribution to the health prospects of its consumers worldwide. Additionally, the peel of these mango fruit could be exploited as a good source to

extract these compounds for the subsequent use in food processing and nutraceutical industries. Increased revenue could also be expected for the growers and traders as these value added mango fruit should fetch a higher price in markets of demand.

A considerable demand has developed in the recent past on novel viable methods to enhance the levels of bioactive compounds in plant products due to increasing interest in their health benefits (Ruiz-García and Gómez-Plaza, 2013). Moreover, a general quest has arisen among consumers, growers, nutritionists, dieticians and retailers regarding the best stage of ripeness to consume fruit and the best stage of maturity to harvest them to obtain the maximum health benefits. Similarly, the interest in the effects of different cultivation practices such as deficit irrigation and the application of plant growth regulators in nutrient enrichment in fruit crops is also in the rise. A thorough understanding of the factors and conditions that can influence the levels of these health-promoting compounds is essential for the manipulation of their concentrations in mango fruit.

The concentration of important health-beneficial terpenoids such as lupeol and carotenoids present in fruit could possibly be increased by the stimulation of terpenoid biosynthesis (McGarvey and Croteau, 1995). Bivalent metal ions Fe^{2+}, Mg^{2+} and/or Mn^{2+} are utilized as cofactors by the enzymes that catalyze the reactions in terpenoid biosynthetic pathway (Fischbach et al., 2000; McGarvey and Croteau, 1995). In agreement with this mechanism, the activity of monoterpene synthase isolated from lemon fruit was significantly higher after the *in vitro* addition of Mn^{2+} as bivalent metal ion cofactor (Lucker et al., 2002). Similarly, the activity of 4-diphosphocytidyl -2-C-methyl-D-erythritol kinase involved in terpenoid biosynthesis was significantly higher when Mg^{2+} was added to isolated cell cultures of tomato fruit (Rohdich et al., 2000). However, the influence of pre-harvest application of these nutrients on the concentration of terpenoidal and other health-promoting compounds in ripe mango fruit warrants to be investigated.

The degree of ripeness significantly influences the level of phenolic compounds in fruit (Belitz et al., 2004). Several climacteric fruit including mango, are harvested at firm mature green stage due to their perishable nature (Lalel et al., 2003a). However, this prolonged shelf life by early harvest could be at the expense of the development

of the full health-beneficial potential of the fruit. The highest concentration of aroma volatile compounds including different important terpenes were recorded in the ripe pulp of 'Kensington Pride' mango fruit harvested at sprung stage (cream pulp, 100% green peel, springy), riper than hard green mature stage (Lalel et al., 2003a). Thus, the influence of the maturity at harvest on the concentrations of lupeol, mangiferin and phenolic acids in the ripe mango fruit also needs investigation.

Mango is a climacteric fruit which undergoes significant physico-chemical changes during the period of ripening, whether still attached to the tree or harvested (Singh et al., 2013). Several changes in the concentrations of polyphenolic compounds have also been reported in mango fruit after the climacteric respiratory peak (Talcott et al., 2005). Thus the process of ripening is apparently a significant determinant of the level of health-promoting compounds in ripe mango fruit. Ethylene is considered as the key controller of these biochemical processes that occur during ripening (Saltveit, 1999; Yang and Hoffman, 1984). The phenolic health-promoting compounds are produced as secondary metabolites in plant tissues (Cisneros-Zevallos, 2003). The phenylpropanoid metabolism which is involved in the production of these secondary metabolites is found to be enhanced by ethylene (Saltveit, 1999). However, the changes in the production of health-promoting compounds in mango fruit during pre- and post-climacteric ripening stages are not yet fully investigated.

The application of different physical and chemical elicitor treatments to increase the concentration of desired nutraceuticals in fruit and vegetables is considered as a promising tool (Ruiz-García and Gómez-Plaza, 2013; Schreiner and Huyskens-Keil, 2006). Low temperature storage and altered gas composition in a storage environment are among the physical elicitor treatments, whilst the application of stress-inducing plant signalling molecules such as methyl jasmonate (MeJA), salicylic acid (SA), nitric oxide (NO) and ethylene are among the reportedly effective chemical elicitor treatments in many fruit and vegetables (Schreiner and Huyskens-Keil, 2006; Zhao et al., 2005). Abiotic stresses such as low temperature and heat as well as stress inducing compounds could induce the biosynthesis of polyphenols such as phenolic acids and xanthones via the shikimic acid pathway as part of the plant defense mechanism (Ruiz-Garcia and Gomez-Plaza, 2013). Elicitor application could possibly overcome the safety concerns, potential ecological imbalance, and high cost

incurred by other biotechnological approaches such as genetic engineering (Flinn and Zavon, 2004) and the high cost and low yields obtained by plant cell culture technology (Zhao et al., 2005). However, the effect of these physical and chemical elicitors on the concentrations of major health-promoting compounds such as lupeol, mangiferin and phenolic acids in ripe mango fruit are unknown.

Thus, the objectives of this research project were;

General objective:

To study the pre- and post-harvest factors and conditions that may regulate the concentrations of health-promoting compounds such as lupeol, mangiferin, phenolic acids (gallic acid, chlorogenic acid, vanillic acid, ferulic acid and caffeic acid), total phenols, total carotenoids, ascorbic acid and total antioxidant capacity in the pulp and peel of ripe mango fruit.

Specific objectives:

1. To investigate the effects of pre-harvest spray application of $FeSO_4$ (Fe^{2+}), $MgSO_4$ (Mg^{2+}) and $MnSO_4$ (Mn^{2+}) solutions on the concentrations of the health promoting compounds such as lupeol, mangiferin, phenolic acids, total phenols, total antioxidants carotenoids, ascorbic acid in the pulp and peel of the ripe mango fruit.

2. To study the effects of different harvest maturities (hard green, sprung stage, half ripe and tree ripe) on the concentrations of the health-promoting compounds in the pulp and or peel of ripe mango fruit after postharvest ripening.

3. To investigate the changes in the concentrations of the health-promoting compounds in the pulp and peel of mango fruit during postharvest ripening.

4. To study the effects of chilling (5 °C) over standard non-chilling (13 °C) low temperature storage on the concentrations of the health-promoting compounds in the pulp and peel of the ripe mango fruit.

5. To examine the effects of postharvest application of chemical elicitors such as methyl jasmonate (MeJA), salicylic acid (SA) and nitric oxide (NO) on the concentrations of health-promoting compounds in the pulp and peel of the ripe mango fruit.

CHAPTER 2

General literature review

2.1 Introduction: Mango, a fruit rich in health-promoting compounds

Mango (*Mangifera indica* L.) has been among the most favoured fruit in the world since ancient history due to its rich flavour and nutritional value. However, in the recent past, its importance as a health promoting commodity has been significantly highlighted (Masibo and He, 2009).

Cardiovascular diseases and cancer are the major reasons of global mortality (WHO, 2017a and WHO 2017b). The estimated number of deaths per year due to cardiovascular diseases alone is over 20 million by 2030 (WHO, 2014). However, several epidemiological studies have presented evidence of the ability of a number of phenolic compounds present in fruit and vegetables in reducing the risk of these chronic ailments (Schreiner and Huyskens-Keil, 2006). Mango is one of such fruit renowned for its rich content of health-promoting compounds which can combat the risk of several degenerative diseases including cardiovascular diseases and different types of cancer (Masibo and He, 2008). Therefore, in the past few decades a considerable amount of literature has been published on the bioactive compounds of mango fruit with special emphasis on their health benefits.

This chapter focuses on: mango production and trade; the position of mango as a nutritious fruit; bioactive compounds and their biosynthesis; health benefits of the bioactive compounds present in mango fruit; the factors that could influence the concentrations of these compounds in mango fruit and; the present understanding of different methods that can be employed to increase the concentrations of these compounds in ripe mango fruit.

2.2 Origin and distribution of mango fruit

Mango belongs to family Anacardiaceae (Bompard, 2009; Singh et al., 2013) and it is possibly evolved in an area including parts of Myanmar, Bangladesh and India

(Bompard, 2009). Even though the natural distribution of mango is limited to tropical Asia (Bompard, 2009); the European settlements in the 15th and 16th centuries might have extended mango cultivation from the centres of domestication to the outside world (Mukherjee and Litz, 2009).

2.3 Production and trade of mango fruit

2.3.1 World statistics

Mango total production (over 42.1 million Mt including mangosteen and guava) ranks fifth among major fruit crops cultivated worldwide, *viz*. banana, citrus, grapes and apples. It is grown over an area of 5.4 million ha in nearly 100 countries in the world (FAOSTAT, 2017). India is in the topmost position among mango growing countries with respect to both quantity of production and the area under cultivation followed by China (Fig. 2.1 and Fig. 2.2).

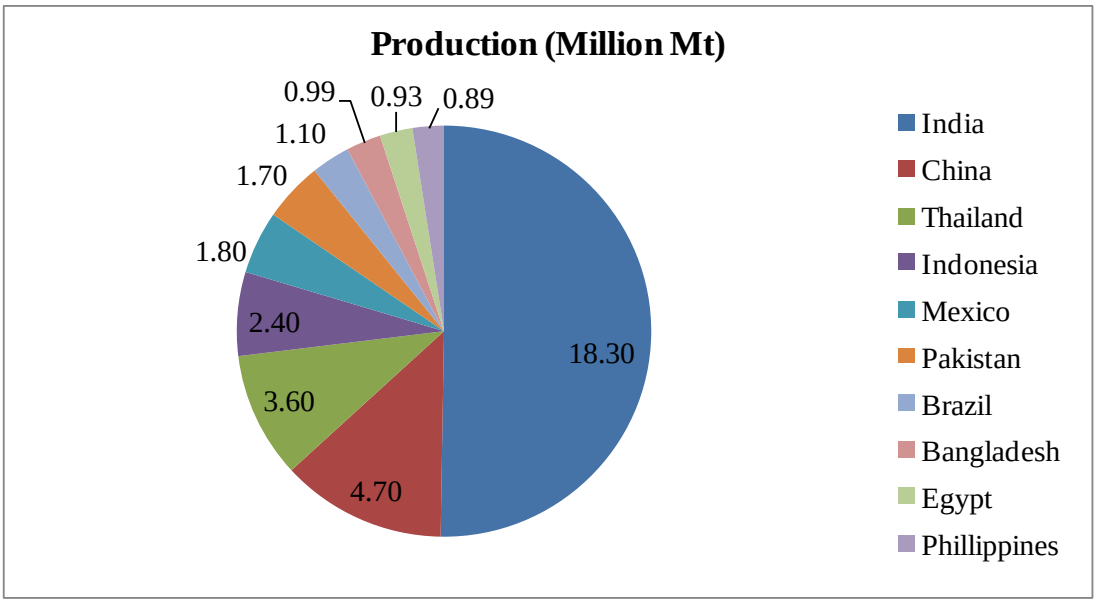


Fig. 2.1 Top mango producing countries in the world and their production (million Mt) (including mangosteen and guava) (FAOSTAT, 2017)

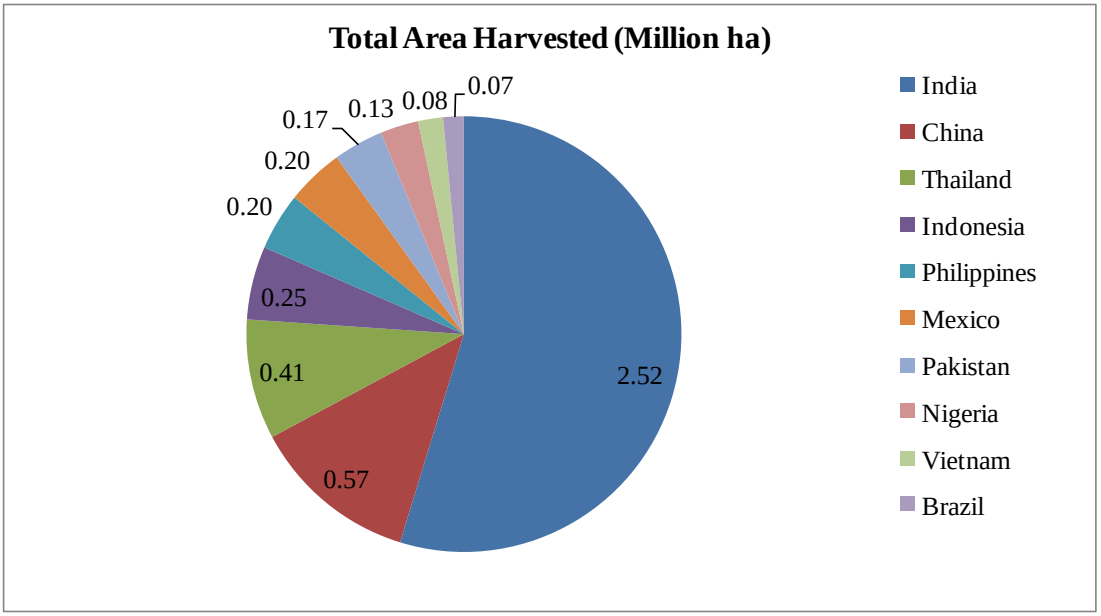


Fig. 2.2 The total area harvested (including mangosteen and guava) in top mango producing countries in the world (FAOSTAT, 2017)

Statistics reveal that the world mango production is increasing steadily over the years, mainly due to the widespread introduction of this crop into new areas outside the traditional centres of mango cultivation including the Western hemisphere (Fig. 2.3). However, it is difficult to provide exact production data or area under cultivation of mango, because the Food and Agriculture Organization (FAO) reports it in combination with the data of mangosteen and guava.

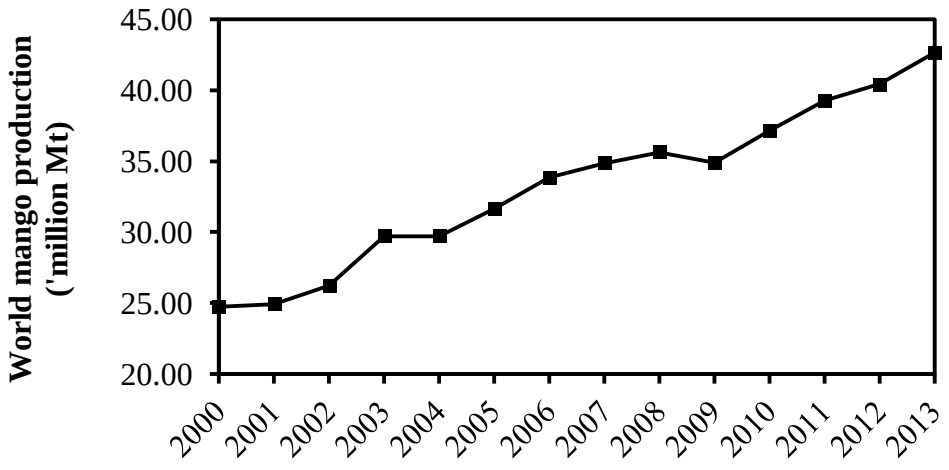


Fig. 2.3 The recent trend of mango production in the world (FAOSTAT, 2017)

In international trade, Mexico enjoys the leading position among the mango exporting countries followed by the Philippines and Pakistan (FAOSTAT, 2017) (Fig. 2.4). The United States of America possesses the largest share in global imports of mangoes followed by the Netherlands (FAOSTAT, 2017) (Figure 2.5). The production and trade statistics over the years clearly show that mango has become one of the most important fruit crops in the world with increasing trends in both production and trade (Fig. 2.6 and Fig. 2.7).

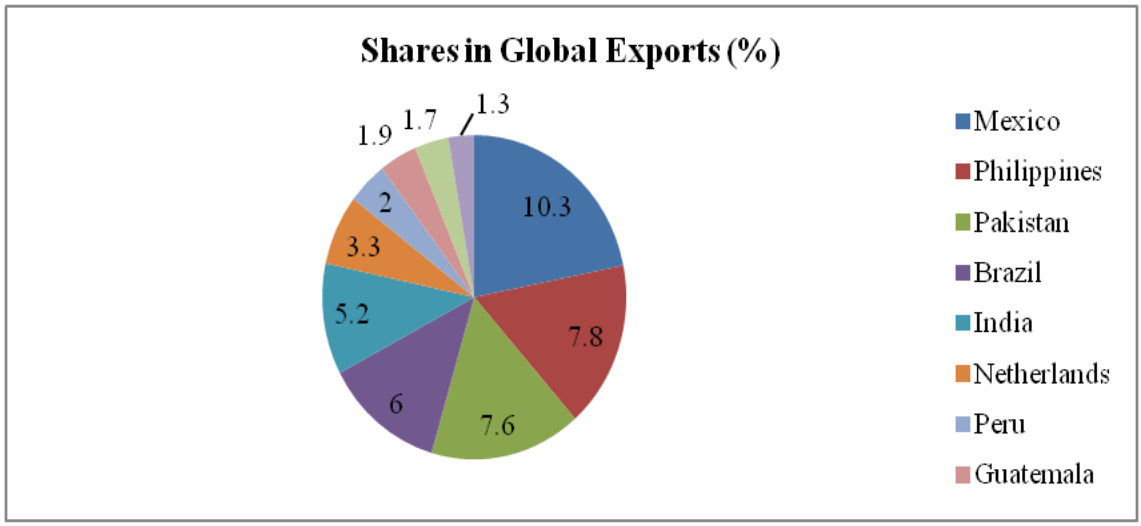


Fig. 2.4 Shares of different countries in global mango exports (%) (FAOSTAT, 2017)

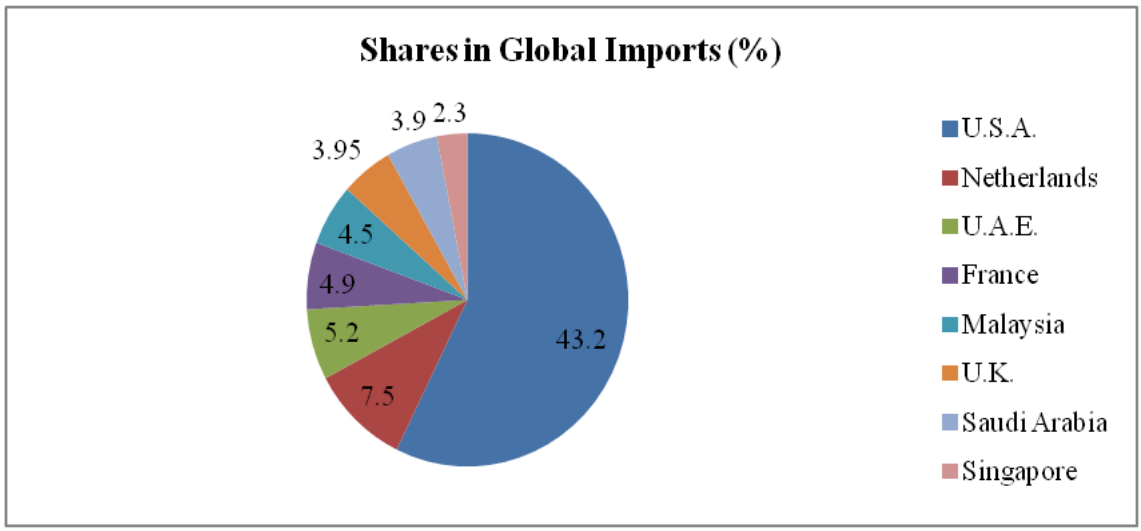


Fig. 2.5 Shares of different countries in global mango imports (%) (FAOSTAT, 2017)

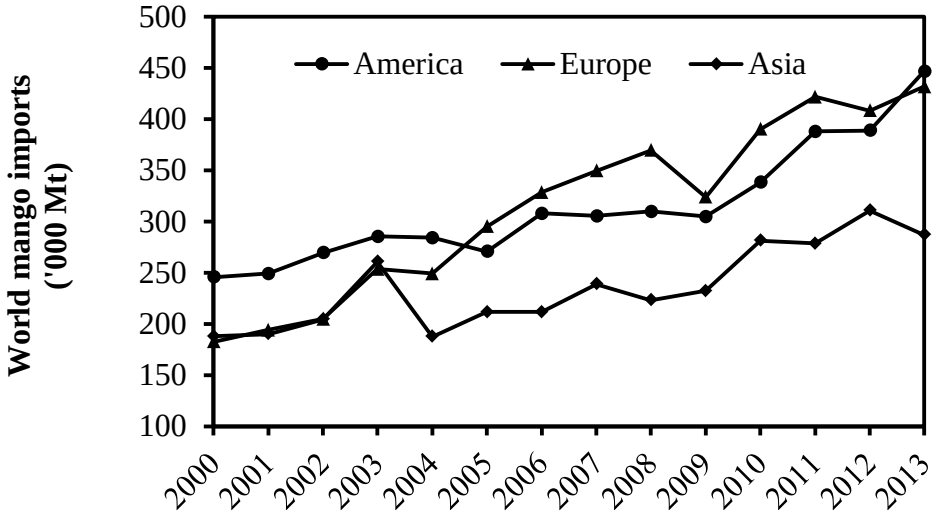


Fig. 2.6 The recent trend of total imports of mango by different regions of the world (FAOSTAT, 2017)

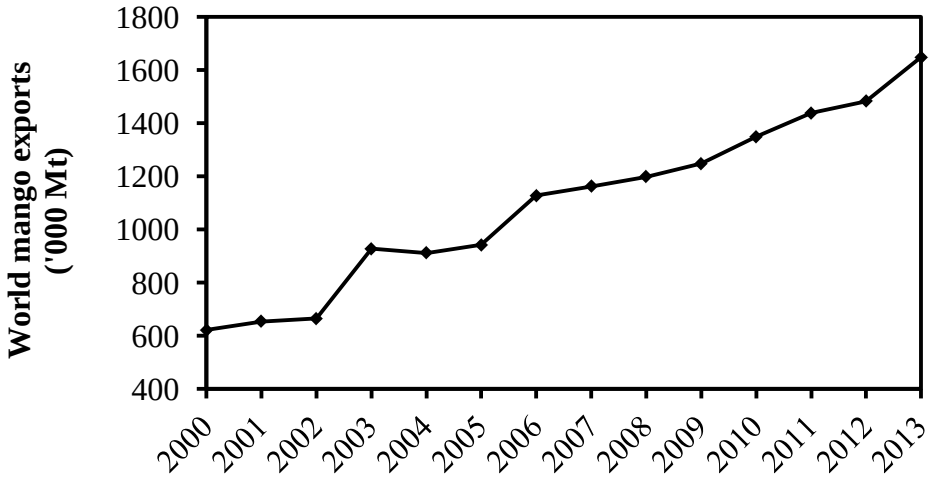


Fig. 2.7 The recent trend of total exports of mango in the world (FAOSTAT, 2017)

2.3.2 Australian statistics

Similarly to the world context, mango is one of the choicest fruit in Australian market. Being a tropical fruit, mangoes are predominantly produced in the northern Australian states with a tropical or sub-tropical climate. The major producing areas include Darwin and Katherine in the Northern Territory (50%) and Mareeba, Bowen and Bundaberg in Queensland (45%). It is also produced in Western Australia (3%)

with small volumes coming from Victoria (1%) and New South Wales (1%) (Fig. 2.8). In 2016, around 63,780 Mt of total mango production was recorded with a total production value of around $210 million (Horticulture Innovation Australia Limited, 2017). The total area of mango production in Australia was 9,500.00 ha (including mangosteen and guava) in 2014 (FAOSTAT, 2017). Australian mangoes are mainly exported to Hong Kong (43%), New Zealand (12%), Singapore (12%), UAE (12%) and Lebanon (6%) (Horticulture Innovation Australia Limited, 2017).

Fig. 2.8 The major mango growing areas in Australia.
Source: http://www.australiafresh.com.au

2.4 Major commercial cultivars of mango

Mango has a wide range of genetic variation (Iyer and Schnell, 2009). Different selection criteria used by different regions and the genetic responses to different environmental conditions have mainly contributed to this diversity (Knight et al., 2009). Likewise, the widespread introduction of mango to non-traditional centres of cultivation has significantly contributed to the recent genetic diversity of this crop (Knight and Schnell, 1994; Schnell et al., 2006; Knight et al., 2009). As a result, there are hundreds of mango cultivars with marked diversity in shape, size, colour, flavour and the composition (Stafford, 1983).

'Ataulfo', 'Tommy Atkins', 'Haden', 'Irwin', 'Keitt', 'Kent', 'Nam Dok Mai', 'Alphonso', 'Dashehari' are some of the most popular commercial mango cultivars in the world (Singh et al., 2013), whilst the Australian mango market is dominated by cultivars 'Kensington Pride' (55%), 'Calypso' (18%), 'R2E2' (10%) and 'Honey Gold' (8%) (Horticulture Innovation Australia Limited, 2017).

2.5 Nutritional aspects of mango fruit

Mango fruit is an important element in the diet of many countries in the world. There is a large volume of published literature describing the chemical composition of mango pulp. In general, mango is a rich source of vitamins and minerals (Table 2.1). It is specifically rich in ascorbic acid (vitamin C), which is known to be one of the most significant vitamins for human nutrition (Masibo and He, 2009). Li et al. (2014) reported that the ascorbic acid content of mango is 9 times higher than that of apple and 4 times higher than that of banana. Moreover, its carotenoid content and α – tocopherol (vitamin E) contents were also very much greater than that of apple showing 10 times and 18 times higher values respectively (Li et al., 2014). Furthermore, mango pulp has been reported to contain high amounts of vitamin A (Table 2.1). The predominant carotenoid in mango is found to be β-carotene, which has been revealed to have high vitamin A activity (Godoy and Rodriguez-Amaya, 1994). However, the location of cultivation, variety, and stage of maturity substantially influence the chemical composition of mango pulp (Tharanathan et al., 2006).

Table 2.1. Nutritional composition of mango pulp

Nutrient	Unit	Amount in 100g of pulp*
Total dietary fibre	g	1.6
Sugars (total)	g	13.66
Sucrose	g	6.97
Glucose	g	2.01
Fructose	g	4.68
Calcium	mg	11
Iron	mg	0.16
Magnesium	mg	10
Phosphorous	mg	14
Potassium	mg	168
Sodium	mg	1
Zinc	mg	0.09
Copper	mg	0.111
Manganese	mg	0.063
Vitamin C	mg	36.4
Thiamin	mg	0.028
Riboflavin	mg	0.038
Niacin	mg	0.669
Pantothenic acid	mg	0.197
Vitamin B-6	mg	0.119
Folate	µg	43
Choline	µg	7.6
Vitamin A	µg	54
β-carotene	µg	640
α-carotene	µg	9
Lycopene	µg	3
Vitamin E	mg	0.9
Vitamin K	µg	4.2

Source: USDA National Nutrient Database for Standard Reference, Release 28 (2016) *Based on analyses of Tommy Atkins, Keitt, Kent, and/or Haden cultivars.

2.6 Health- promoting compounds

Traditionally, fruit have been recognized as a major source of vital dietary micronutrients and fibres. However, in the past few decades mango fruit has been acknowledged as an important source of phytochemicals (Haminiuk et al., 2012). Consequently, a large and growing body of literature has reported the types and concentrations of phytochemicals in mango as it is one of the most consumed fruit in the world. Phenolic compounds and terpenoids are among the major groups of compounds with health benefits.

A number of studies have revealed that virtually every part of mango tree, *viz*. pulp, peel, and seed of mango fruit, extracts from bark, leaves and flowers are a good source of beneficial phytochemicals (Ajila et al., 2007; Masibo and He 2009; Kim et al., 2010a). Mango fruit is found to be rich in dietary antioxidants, such as ascorbic acid, carotenoids and phenolic compounds (Ma et al., 2011) and it has shown a high therapeutic value mainly due to its significant antioxidant capacity (Kim et al., 2007).

2.6.1 Phenolic health-promoting compounds

A substantial number of bioactive compounds present in plant materials are classified as phenolic compounds (Pierson et al., 2012). A common feature of all phenolic compounds is the presence of an aromatic ring which is directly linked to one or more hydroxyl groups (Fig. 2.9). Compounds with more than one hydroxyl groups attached to one or more aromatic rings (benzene rings) are called polyphenols (Vermerris and Nicholson, 2006).

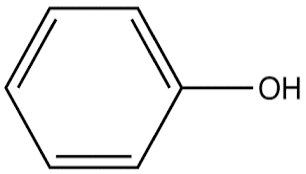


Fig. 2.9 Chemical structure of phenol

2.6.1.1 Classification of phenolic compounds

Phenolic compounds are mainly classified based on the number of phenol rings they contain (Vermerris and Nicholson, 2006). They are generally divided as simple phenols, phenolic acids, flavonoids, tannins, xanthones, stilbenes, lignans and lignins (Naczk and Shahidi, 2004; Manach et al., 2004; Vermerris and Nicholson, 2006). Flavonoids are the main bioactive compounds found in fruit and are divided into six subclasses: flavonols, flavanones, isoflavones, flavan-3-ols, flavones and anthocyanins (Robbins, 2003).

Phenolic acids are aromatic secondary plant metabolites which derived from two parent structures called hydroxybenzoic acid (Fig. 2.10) and hydroxycinnamic acid (Fig.2.11) (Khoddami et al., 2013). Thus, they are basically divided into two groups, *viz.* hydroxybenzoic acids and cinnamic acids. Gallic acid, protocatechuic acid and vanillic acid are among the major hydroxybenzoic acids whilst, *p*- coumaric acid, caffeic acid, ferulic acid and sinapic acid are classified as cinnamic acids. Cinnamic acids are commonly found in plants as esters of quinic acid, shikimic acid, and tartaric acid. Chlorogenic acid which possesses significant health benefits is an ester of caffeic caid and quinic acid.

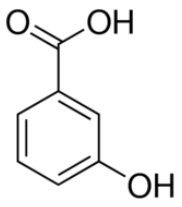


Fig. 2.10 Hydroxybenzoic acid

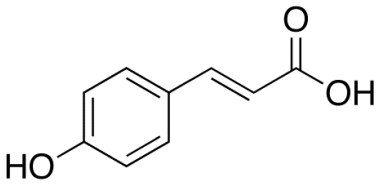


Fig. 2.11 Hydroxycinnamic acid

2.6.1.2 Bioactivities of phenolic compounds

Phenolic compounds play a crucial role in the visual appearance, flavour and health benefits of fruit and vegetables (Tomás-Barberán and Espin, 2001). Their strong ability to act as antioxidants is the major reason for their health properties (Tsao, 2010).

Antioxidants are classified into different groups based on their mode of action, *viz*. oxygen scavengers, free radical terminators and chelators of metal ions that can catalyse lipid oxidation (Shahidi et al., 2009). For those phenolic compounds classified under the category of free radical terminators, the hydroxyl group attached to the aromatic ring has the ability to donate either hydrogen or electrons to scavenge free radicals. They impede lipid oxidation by rapid donation of hydrogen to lipid radicals (Shahidi et al., 2009). This property enables them to protect human cells against oxidative damages such as lipid peroxidation and DNA damage (Masibo and He, 2008).

Flavonoids, phenolic acids, anthocyanins and tannins, present in fruit have demonstrated their significant antioxidant potential and health benefits (de Pascual-Teresa et al., 2010). Several epidemiological studies have revealed that the frequent consumption of plant foods having dietary phenolics can contribute to human health by reducing the risk of degenerative diseases (Naczk and Shahidi, 2004). This growing evidence of health benefits has created new hope for health prospects, through polyphenol rich food and nutritional supplements (Scalbert et al., 2005).

2.7 Terpenoids

Terpenoids are considered as the largest family of natural compounds. This group of compounds comprises of several primary and secondary metabolites with a significant importance for the survival of plants and with biological properties beneficial to mankind (Aharoni et al., 2005). In addition to their several commercial uses as flavours, fragrances and colouring agents in food processing industry and industrial uses as adhesives, coatings and agrochemicals, terpenoids are known for their health beneficial properties (Aharoni et al., 2005; McGarvey and Croteau, 1995).

Terpenoids are derived by the recurring fusion of five-carbon units called ‘isoprene units’ (McGarvey and Croteau, 1995). Thus, they are divided in to several groups based on the number of isoprene units they possess; namely, hemiterpenes (C_5, single isoprene unit), monoterpenes (C_{10}, two isoprene units), sesquiterpenes (C_{15}, three

isoprene units), diterpenes (C_{20}, four isoprene units), triterpenes (C_{25}, five isoprene units), tetraterpenes (C_{30}, six isoprene units) and polyterpenes (> C_{40}, > 8 isoprene units) (Croteau et al., 2000). Plant growth regulators (cytokinins, gibberellic acid and abscisic acid), photosynthetic pigments (carotenoids and chlorophyll) and ubiquinones used in respiration are some of the most important primary terpenoid metabolites (Aharoni et al., 2005). Monoterpenes, sesquiterpenes, diterpenes and triterpenes are considered as secondary metabolites (Aharoni et al., 2005).

Monoterpenes comprise a major fraction of aroma volatile compounds in fruit such as mango (Lalel et al., 2003a), floral fragrance and of essential oils in plants (Croteau et al., 2000). Sesquiterpenes are also found in mango fruit aroma volatile compounds (Lalel et al., 2003a) and essential oils in plants and a number of sesquiterpenoids are found in antimicrobial compounds produced by plants as pathogen defence (Croteau et al., 2000). Diterpenes include phytol, a side chain of chlorophyll molecule and several other pharmacologically important compounds such as retinol (Croteau et al., 2000), whilst triterpenes include plant hormones, various membrane components, plant waxes (Croteau et al., 2000) and anti-carcinogenic bioactive compounds such as lupeol (Saleem, 2009). Tetraterpenes which is the most common of all terpene groups is consisted of carotenoid pigments (Croteau et al., 2000).

2.8 Major health-promoting compounds present in mango fruit and their importance

Lupeol, mangiferin, gallic acid, chlorogenic acid, vanillic acid, caffeic acid, ferulic acid, protocatechuic acid, anthocyanins, quercetin and kaempferol, are among the secondary compounds present in mango fruit with significant beneficial bioactive properties for humans (Masibo and He, 2008; Palafox-Carlos et al., 2012a; Saleem, 2009). However, the concentrations of these health-promoting compounds present in mango vary in different parts of the fruit such as pulp, peel and seed (Masibo and He, 2008). The concentration of lupeol in ‘Ataulfo’ mango was 1to 4 times more in the peel than pulp depending on the harvest maturity (Ruiz-Montañez et al., 2014), whilst it was 1to 40 times more in North Indian mangoes depending on the cultivar (Srivatava et al., 2015).

2.8.1 Lupeol

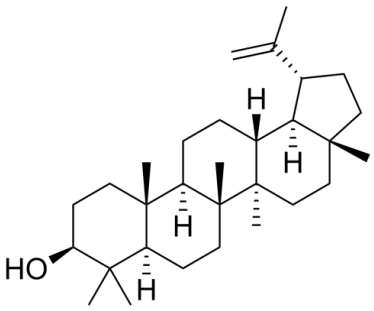


Fig. 2.12 The chemical structure of lupeol (Source: https://www.sigmaaldrich.com)

Lupeol is a naturally occurring pentacyclic triterpine that present in various plant parts in different concentrations. Mango pulp, grape, hazelnut, olive oil, carrot root, cucumber, soybean and cabbage are found to be rich sources of this compound (Gallo and Sarachine, 2009; Saleem, 2009; Syed and Mukhtar, 2011).

The ability of lupeol to selectively target diseased human cells is well known (Saleem, 2009). Lupeol is also known for its ability to interact with multiple molecular targets to help control carcinogenesis (Gallo and Sarachine, 2009).

A study on mouse prostrate cells demonstrated that lupeol could prevent development of cancer cells and eliminate existing cancer cells through induction of apoptosis (Prasad et al., 2008). Similar results were found in the induction of apoptosis in human prostate cancer cells (Prasad et al., 2008). Further, its efficacy in controlling colorectal cancer cells (Tarapore et al., 2013), bone marrow cancer cells (Prasad et al., 2008) and cutaneous melanoma (Syed and Mukhtar, 2011) were also reported.

Various *in vitro* and preclinical animal studies suggest that lupeol has a potential to act as an anti-inflammatory, anti-invasive, anti-angiogenic and cholesterol lowering agent. Furthermore, it has shown its capability as an anti-arthritic, anti-microbial, anti-protozoal and anti- diabetic agent (Siddique and Saleem, 2011).

2.8.2 Mangiferin

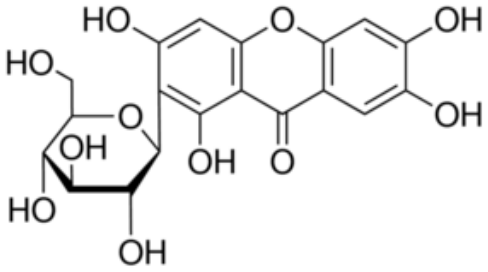


Fig. 2.13 The chemical structure of mangiferin (Source: https://www.sigmaaldrich.com)

Mangiferin (C-2-β-D-glucopyranosyl-1, 3, 6, 7-tetrahydroxyxanthone) is a glucosyl xanthone present in several parts of mango tree including the fruit (Masibo and He, 2009). It has been found to possess a wide array of pharmacological potentials such as: antioxidant, anticancer, antimicrobial, antiatherosclerotic, antiallergenic, anti-inflammatory and analgestic among many others. Many studies have revealed that the majority of the health beneficial properties of mango extract have been ascribed to this polyphenolic compound (Masibo and He, 2009). Further, mangiferin is capable of showing antioxidant potential at extremely low concentrations (Martinez et al., 2001).

Iron is a mediator for free radical effects in several chronic degenerative diseases such as ischemic heart disease and cancer (Puccio and Kœnig, 2002). Iron-complexing ability of mangiferin, which was reported in many studies, can reduce this iron-induced oxidative damage (Puccio and Kœnig, 2002; Halliwell and Gutteridge, 1986). Furthermore, it has a confirmed ability of reducing the progression of degenerative diseases including Parkinson's disease, in which oxidative stress plays a crucial role (Halliwell, 2006). Moreover, mangiferin has shown potential to ameliorate the oxidative stress results in neurodegenerative disorders due to its ability to pass through blood-brain barrier (Martinez et al., 2001). It has been suggested that mangiferin also protects erythrocytes and red blood cells from reactive oxygen species production (Pawlak et al., 1998; Rodriguez et al., 2006).

Mangiferin was found to significantly reduce plasma total cholesterol, triglycerides and low density lipoprotein (LDL) in diabetic rats (Muruganandan et al., 2005) and blood glucose level by inhibiting the glucose absorption from the intestine (Yoshikawa et al., 2001; Aderibigbe et al., 2001). Besides, mangiferin could inhibit body weight gain in experimental rats showing a potential in its usage in designing novel food products for special dietary needs for obese people (Yoshimi et al., 2001).

2.8.3 Phenolic acids

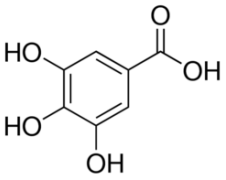
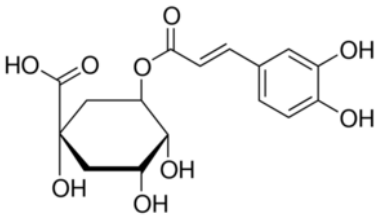
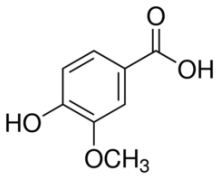
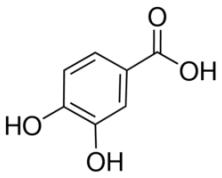
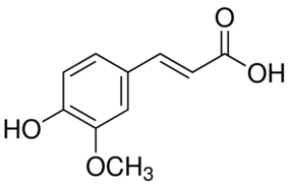
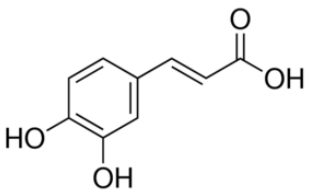
Mango fruit is rich in several phenolic acids which are known for their therapeutic potential (Masibo and He, 2009; Palafox-Carlos et al., 2012a; Palafox-Carlos et al., 2012b) (Table2.1). Gallic acid, chlorogenic acid and vanillic acid are among the major phenolic acids in mango fruit, whilst protocatechuic acid, ferulic and caffeic acids also present in lower concentrations (Palafox-Carlos et al., 2012a; Vithana et al., 2017).

Gallic acid (3, 4, 5-trihydroxybenzoic acid) was identified as one of the major phenolic acids present in mango fruit (Kim et al., 2007; Palafox-Carlos et al., 2012a; Vithana et al., 2017). It has a strong antioxidant potential in emulsion or lipid systems (Yen et al., 2002; Madsen and Bertelsen, 1995). Gallic acid has demonstrated its potential in inhibiting carcinogenesis in several animal models and in *in vitro* cancerous cell lines (Verma et al., 2013). Ho et al. (2013) suggested that gallic acid can be considered as a potential agent to treat gastric cancer, whilst Chen et al. (2009) suggested that gallic acid has the potential to be developed into an anti-prostate cancer drug. Gallic acid has shown a similar potential in controlling human leukemia (Reddy et al., 2012), bone cancer (Liao et al., 2012), breast cancer (Parihar et al., 2012) and lung cancer (Ji et al., 2009) in cell culture studies. In several studies, gallic acid was identified as the principle constituent in plant extracts that cause growth inhibition and apoptotic death of DU145 human prostate carcinoma cells (Veluri et al., 2006; Chen et al., 2009). Thus, Chen et al. (2009) suggested that gallic acid has the potential to be developed into an anti-prostate cancer drug. Moreover, Inoue et al. (1995) reported that gallic acid was cytotoxic against all cancer cells that they examined.

Based on several *in vivo* and *in vitro* studies; chlorogenic acid, the ester of caffeic and quinic acid was also found to be capable of exhibiting important antioxidant and anti-carcinogenic activities (Farah et al., 2008). It has demonstrated its ability in protecting healthy cells against apoptosis induced by oxidative stress by suppressing reactive oxygen species (Li et al., 2012). Further, Cho et al. (2010) reported that chlorogenic acid possesses anti-obesity property and could improve lipid metabolism in obese mice. The ability of chlorogenic acid in suppressing asthma (Kim et al., 2010b), lipopolysaccharide-induced acute lung injury in mice (Zhang et al., 2010), liver inflammation and fibrosis (Shi et al., 2013), diabetes (Ong et al., 2013) and ischemia/reperfusion injury in rat liver (Yun et al., 2012) have also been reported. Vanillic acid (4-hydroxy-3-methoxy benzoic acid) has also shown several beneficial properties (Zheng and Wang, 2001). Kumar et al. (2014) reported that, vanillic acid was capable of normalizing hypertension and left ventricular function in experimentally induced hypertensive rats and thus has the potential to be developed as a functional drug in the management of hypertension: the most common cardiovascular disorder. The protective role of vanillic acid in isoproterenol induced cardiotoxic rats due to its free radical scavenging and anti-inflammatory properties was also reported (Prince et al., 2011). Furthermore, Sindhu et al. (2015) also reported that vanillic acid can be used in combination with cancer chemotherapy.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) had been identified as a principal compound with high therapeutic properties in traditional Chinese medicine (Ou and Kwok, 2004). This phenolic acid is also endowed with many physiological functions including antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis, and anti-cancer activities. It also plays a protective role against coronary diseases and lowers cholesterol (Ou and Kwok, 2004). Kanski et al., (2002) reported that ferulic acid could greatly reduce the free radical damage in neuronal cell systems and therefore; possesses a significant therapeutic potential against neurodegenerative disorders such as Alzheimer disease. Caffeic acid (3, 4-dihydroxycinnamic acid) is another phenolic acid known for its strong antioxidant potential (Gülçin, 2006) and is also recognized as an effective anti-diabetic agent (Jung et al., 2006). Moreover, caffeic acid has exhibited its ability in protecting pBR322 plasmid DNA against the mutagenic and toxic effects of UV radiation and hydrogen peroxide (H_2O_2) (Sevgi et al., 2015).

Table. 2.2 Phenolic acids present in mango fruit (Palafox-Carlos et al., 2012a; Vithana et al., 2017)

Phenolic acid	Chemical structure*
Gallic acid	
Chlorogenic acid	
Vanillic acid	
Protocatechuic acid	
Ferulic acid	
Caffeic acid	

(*Source: https://www.sigmaaldrich.com)

2.9 Factors affecting the levels of health-promoting compounds (lupeol, mangiferin, phenolic acids, ascorbic acid, carotenoids and antioxidants) in mango fruit

2.9.1 Effect of the genotype

Mango has a wide range of genetic variation (Iyer and Schnell, 2009). There are hundreds of mango cultivars with distinct visual diversity in shape, size and colour and internal differences in flavour and the nutrient composition (Stafford, 1983). In addition to these differences, a significant diversity in the concentrations of bioactive compounds has also been reported in mango fruit.

According to Srivastava et al. (2015), a significant variation has been observed in the concentration of lupeol in the pulp and peel among four Indian mango cultivars: 'Bombay Green', 'Dashehari', 'Langra' and 'Chausa'. A significantly high concentration of lupeol was found in both the pulp and peel of 'Dashehari' mango fruit followed by 'Langra', whilst a significantly lower amount was noted in the fruit of 'Chausa'. Similarly, Ruiz-Montanez et al. (2014) reported that the concentration of lupeol was influenced by the cultivar in a study carried out using 'Ataulfo' and native mango fruit.

The concentration of mangiferin was significantly different in four cultivars of Brazilian mangoes; recording 2.9 mg kg^{-1} in 'Haden', 2.2 mg kg^{-1} in 'Tommy Atkins', 12.4 mg kg^{-1} in 'Uba' on dry weight basis, whilst it was not detected in the fruit of cultivar 'Palmer' (Ribeiro et al., 2008). Srivastava et al. (2015) reported that the concentration of mangiferin was the highest in the pulp of 'Bombay Green' mango fruit compared to 'Dashehari', 'Langra' and 'Chausa', while it was the highest in the peel of 'Langra' fruit compared to other three. Similar observations were recorded in the concentrations of flavonol-*O*-glycosides in these cultivars, thus providing evidence of the genotypic influence in the concentration of secondary metabolites (Ribeiro et al., 2008). Further, the concentration of mangiferin in the peel ranged from 300 – 1300 mg kg^{-1} in dry weight basis among 'Chok Anan', 'Tommy

Atkins', 'Maha Chanock' and 'Kaew' mango fruit recording significantly lower concentrations in 'Haden' and 'Kent' (Berardini et al., 2005a).

Manthey and Perkins-Veazie (2009) reported that significant varietal differences were observed in the concentrations of different health-promoting compounds among five varieties of mango fruit evaluated in multiple harvests. In this study, the concentration of ascorbic acid was ranged between 11.0 – 134.0 mg $100g^{-1}$ of pulp puree, whilst the concentration of β-carotene ranged between 5.0 – 30.0 mg kg^{-1} in 'Tommy Atkins', 'Kent', 'Keitt', 'Haden' and 'Ataulfo' mango fruit. Moreover, a significantly high concentration of total phenolics was noted in the pulp of 'Ataulfo' mango fruit {166.7 mg gallic acid equivalent (GAE) $100g^{-1}$}, whilst the other four cultivars recorded an average of 31.2 mg GAE $100g^{-1}$ in fresh weight basis. The major phenolic acids identified in 'Ataulfo' mango fruit were chlorogenic acid, gallic acid, vanillic acid in the order of relative abundance (Palafox-Carlos et al., 2012a), whereas, gallic acid was the major phenolic acid present in the pulp of 'Tommy Atkins' mango fruit (Kim et al., 2007).

According to Ma et al. (2011), the concentration of total phenols in the pulp varied from 8.8 to 193.4 mg GAE 100 g^{-1} in fresh weight basis among eight cultivars of mango fruit they studied, 'Tainong' having the highest and 'Guifei' the lowest. The total flavonoid content of the pulp was also the highest in 'Tainong' mango fruit (90.9 mg rutin 100 g^{-1}), whilst the fruit of 'Guifei' having the lowest content (6.28 rutin 100 g^{-1}). The concentration of ascorbic acid in the pulp was in the range between 19.8 and 34.6 mg 100 g^{-1} in fresh weight basis among the fruit of these cultivars, 'Mallika' having the highest (Ma et al., 2011). Li, et al. (2014) reported that the concentrations of ascorbic acid, total polyphenols and total flavonoids were significantly high in green peel mango fruit compared to red peel or yellow peel mangoes of 11 different cultivars. However, the levels of nutraceuticals in major Australian mango cultivars are not yet fully investigated.

2.9.2 Effect of the degree of ripeness

The degree of ripeness has shown a marked influence in the concentration of bioactive compounds in mango fruit. It was reported that the major phenolic compounds in 'Ataulfo' mango pulp, tended to increase with fruit ripening (Palafox-Carlos et al., 2012b). The highest concentration of total phenols was recorded in the pulp of 'Ataulfo' mango fruit harvested at 20-30% and 70-80% yellow surface stages when compared with the fruit harvested at 0-10% and 100% yellow surface stages (Palafox-Carlos et al., 2012b). Similarly, the concentrations of chlorogenic acid and vanillic acid were significantly high in the fruit harvested at 71-100% yellow peel stage (Palafox-Carlos et al., 2012a).

According to Ruiz-Montanez et al. (2014), significantly higher concentrations of lupeol and mangiferin were noted in the pulp and peel of 'Ataulfo' mango fruit harvested at consumption maturity stage when compared with the fruit harvested at physiological maturity stage. The concentration of lupeol in the peel of 'Dashehari' mango fruit has also shown a significant increase during ripening (Srivastava et al., 2015).

Concentration of β-Carotene in the pulp was significantly increased in 'Tommy Atkins' mango fruit during ripening (Gil et al., 2000). The concentration of total carotenoids also increased in the pulp of 'Tommy Atkins' and 'Keitt' from mature green to ripe stage including all trans- β-carotene, all trans- violaxanthin, and 9-cis-violaxanthin (Mercadante and Rodriguez-Amaya, 1998).

However, the influence of harvest maturity on the concentrations of lupeol, mangiferin, phenolic acids and other health-promoting compounds after postharvest ripening of mango fruit is yet unknown.

2.9.3 Effect of low temperature storage

Although many studies have investigated the effect of low temperature storage on the content of bioactive compounds in different fruit and vegetables, published data are

rather limited in mango fruit. An overall increase in the concentration of ascorbic acid in the pulp of cold stored (at 7 °C) 'Alphonso' mango fruit was earlier reported indicating an enhanced production or reduced loss of this compound during low temperature storage (Thomas and Janave, 1975). A net increase in the concentration of ascorbic acid compared to the initial content was recorded in the pulp of 'Kent' mango fruit stored below 13 °C claiming an increased production under low temperature storage (Vazquez-Salinas and Lakshminarayana, 1985).

An evaluation of the combined effect of the ripeness stage and cold storage on the content of bioactive compounds revealed that the total phenols and carotene concentrations continued to increase in the pulp of tree ripe 'Irwin' mango fruit stored for 20 d at 5 °C (Shivashankara et al., 2004). The exposure of fruit and vegetables to low temperature stress during storage is considered as a physical elicitor treatment which triggers the production of desired phenolic compounds (Schreiner and Huyskens-Keil, 2006). The biosynthetic pathways of both terpenoids and phenols are activated after an elicitor treatment by inducing the activity of the enzyme, phenylalanine ammonia-lyase (PAL) (Cisneros-Zevallos, 2003; Ruiz-García and Gómez-Plaza, 2013). The concentrations of ferulic acid and caffeic acid were better maintained or increased during storage at 5 °C in papaya cv. 'Maradol', another popular tropical fruit (Rivera-Pastrana et al., 2010). Thus, an increase in the concentrations of phenolic and other health-promoting compounds in mango fruit could also be anticipated under low temperature stress but warrants to be investigated.

2.9.4 Effect of climate, soil composition and the geographical location of cultivation

Investigations on the effect of climatic and soil related factors on the concentrations of health-promoting compounds in mango fruit are rather scarce. According to Manthey and Perkins-Veazie (2009), the country of origin with differences in soil and climate had a small but significant influence on the concentrations of β-carotene, ascorbic acid and total phenolics in five different cultivars of mango grown in four countries. Palafox-Carlos et al. (2012b) reported that, the concentrations of phenolic compounds present in 'Ataulfo' mango fruit grown in Tepic, Nayarit, Mexico were

different from the reported data on 'Ataulfo' mangoes cultivated in Chiapas State, Mexico (Robles-Sánchez et al., 2009).

However, drawing major conclusions from published research on the effect of climate and location on the content of bioactive compounds in fruit is rather difficult due to inconclusiveness and controversy of the findings. The magnitude of the effect of these factors on the concentration of health-promoting compounds is possibly dependent on the type of the fruit and type of the compound. Zheng et al. (2012) reported that the concentrations of ascorbic and quinic acid in sea buckthorn berry was hardly affected by the latitude and weather conditions, whereas, significant differences in total phenol content was detected among currant cultivars grown in different latitudes and weather. Similar, compositional response of black currant berries to latitude and weather conditions were reported by Yang et al. (2013) and Vagiri et al. (2013) revealing significant effects of location and year on the concentration of these compounds.

2.9.5 Effect of postharvest heat treatment

Exposure to thermal quarantine treatments such as hot water treatment to prevent the introduction of invasive pests is a must when mangoes are imported to countries such as the United States (Kim et al., 2009). However, this might affect the concentrations of bioactive compounds in the treated fruit. The concentrations of gallic acid, gallotannins and total soluble phenolics in mature green 'Tommy Atkins' mango fruit were decreased within 2 h of treatment due to prolonged hot water treatment (46.1 °C, 110 min) compared to untreated fruit (Kim et al., 2009). However, when stored for 4 days, the concentration of total soluble phenolics in all hot water-treated fruit decreased regardless of the duration of treatment (70 min or 110 min) whereas only slight differences were observed in gallic acid and gallotannin concentrations.

Similarly, the concentrations of gallic acid and several hydrolysable tannins in the pulp of 'Tommy Atkins' mango fruit subjected to hot water treatment (46 °C for 75 min) prior to storage for 2 weeks at 10 °C under controlled atmospheric conditions were unaffected by the hot water treatment, while total polyphenolics decreased

throughout fruit ripening, regardless of hot water treatment or storage atmosphere (Kim et al., 2007).

2.9.6 Role of ethylene on biosynthesis of health-promoting compounds

Role of ethylene in biosynthesis of phenolic and terpenoid health-promoting compounds in mango fruit is yet unknown. The phenolic compounds are synthesized via shikimic acid pathway, which is initiated by the deamination of phenylalanine by phenylalanine ammonia lyase (PAL) (Ruiz-García and Gómez-Plaza, 2013). Saltveit, (1999) reported that, the phenylpropanoid metabolism is enhanced by ethylene. Besides, Watkins (2006) reported that the activity of PAL and the concentration of total phenols in several fruit are reduced with the exposure to ethylene antagonist 1-methylcyclopropene (1-MCP). Moreover, the concentrations of total phenols and different phenolic acids, *viz*. chlorogenic acid, caffeic acid and ferulic acid were significantly decreased in 'Black Amber' plum fruit with pre-harvest application of ethylene biosynthesis inhibitor such as aminoethoxyvinylglycine (AVG) (Ozturk et al., 2012). However, according to Heredia and Cisneros-Zevallos (2009) the effect of exogenous application of ethylene on the concentration of total phenolics in fruit and vegetables is tissue dependent. Even though the activity of PAL enzyme was increased, the total phenol content was increased only in carrots, green beans and lettuce by the exogenous application of ethylene among 20 fruit and vegetables they analysed.

Kita et al. (2007) have reported that the expression of carotenogenetic gene, phytoene synthase (*PmPSY-1*) and carotenoid accumulation in two Japanese apricot cultivars were increased by the exogenous application of ethylene. Similar results were reported on the effect of exogenous ethylene by Rodrigo and Zacarias (2007) on carotenoid accumulation and the expression of carotenogenic genes in the exocarp (flavedo) of orange fruit.

2.10 Regulation of the concentration of health-promoting compounds in mango fruit

The rediscovery of the historic bond between plant products and human health has instigated a marked growth in the interest of botanical therapeutics, plant-based pharmaceutical products, dietary supplements and functional food among health conscious consumers worldwide (Raskin et al., 2002). Many of the plant derived products with significant medicinal properties are anticipated to complement the conventional medicines in near future and thereby add a significant value to agricultural produce (Raskin et al., 2002).

Due to the ever increasing evidence of an inverse relationship between the regular consumption of fruit and vegetables and chronic degenerative diseases such as cardiovascular diseases and cancer, different methods to improve the content of bioactive compounds in plant products have been developed (Ruiz-García and Gómez-Plaza, 2013). Therefore; in the past few decades, several methods including simple cultural practices such as pruning and fruit thinning to complex methods such as genetic engineering and plant cell culture have been investigated and practiced with the aim of increasing the concentration of bioactive compounds in different fruit and vegetables (Ruiz-García and Gómez-Plaza, 2013).

Phenolic compounds and terpenoids are among the key contributors to the health benefits of fruit and vegetables. Thus, a good understanding of their biosynthetic pathways and the factors that could influence their biosynthesis and degradation would be of utmost importance in developing effective and efficient methods to regulate the concentrations of desired health-promoting compounds in mango fruit.

2.10.1 Biosynthesis of phenolic health-promoting compounds

Most of these phenolic compounds are classified as secondary metabolites that have a large variety of structures and functions (Haminiuk et al., 2012), and are synthesized in plants during normal growth and development or when they are subjected to biotic or abiotic stresses. Biosynthesis of phenolic compounds in plants

as secondary metabolites occurs via different pathways. Generally phenolic compounds are biosynthesised from the intermediates of carbohydrate metabolism via the shikimic acid pathway which is predominately found in plastids (Seigler, 1998). The two starting compounds of shikimic acid pathway are erythrose - 4 - phosphate and phosphoenol pyruvate derived from carbohydrate metabolism during photosynthesis (Seigler, 1998). Several phenolic secondary metabolites are then synthesized from these precursors in multiple steps (Seigler, 1998) (Fig. 2.14). Phenylalanine ammonia lyase (PAL) is one of the key enzymes in shikimic acid pathway which is responsible for the biosynthesis of phenolic acids (Tsao, 2010).

Derivation of erythrose - 4- phosphate (Fig. 2.15) and phosphoenol pyruvate (Fig. 2.16) from carbohydrates, production of shikimic acid (Fig. 2.17) and chorismic acid (Fig. 2.18) and the conversion of chorismate to other products can be considered as the major steps of shikimic acid pathway (Seigler, 1998). The synthesis of phenylalanine (Fig. 2.19) from chorismic acid is one of the crucial steps of polyphenol biosynthesis as polyphenols are basically synthesised from phenylalanine. The deamination of phenylalanine by the enzyme phenylalanine ammonia-lyase (PAL) is considered as the initial step of phenolic acid biosynthesis (Ruiz-García and Gómez-Plaza, 2013). These phenolic acids, mainly cinnamic acid and its derivatives then play a key role in the synthesis of flavonoids, lignin and several other phenolic compounds (Seigler, 1998).

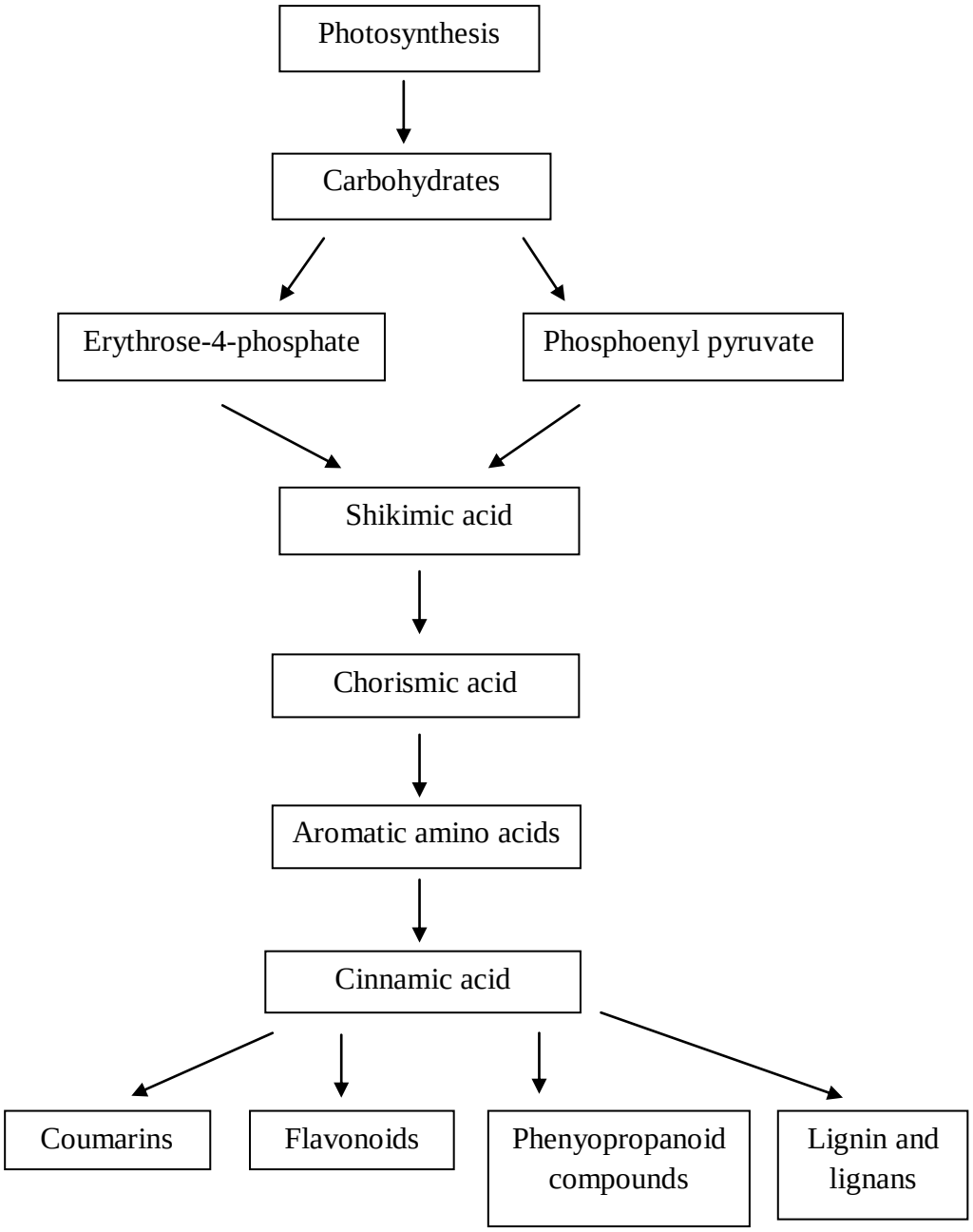


Fig. 2.14 Basic steps of shikimic acid pathway of phenolic biosynthesis modified from Seigler (1998)

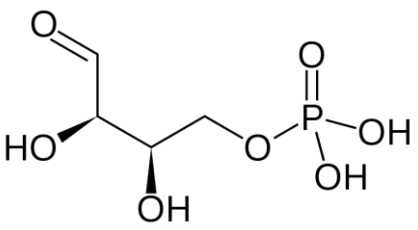


Fig. 2.15 Erythrose-4-phosphate

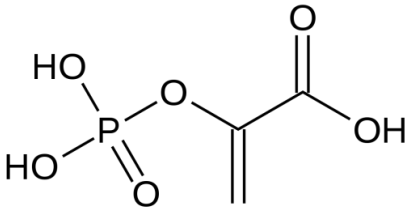


Fig. 2.16 Phosphoenol pyruvate

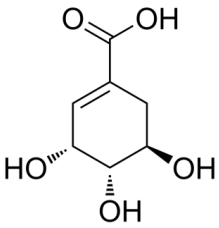


Fig. 2.17 Shikimic acid

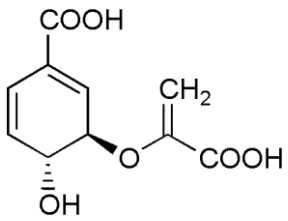


Fig.2.18 Chorismic acid

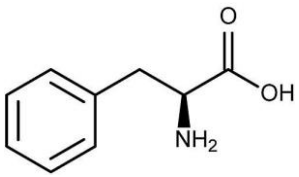


Fig. 2.19 Phenylalanine

(Source: https://www.sigmaaldrich.com)

2.10.2 Biosynthesis of terpenoids

Terpenoids are biosynthesised via mevalonate pathway or 2-C-methyl-D-erythritol-4-phophate (MEP) pathway (Aharoni et al., 2005; McGarvey and Croteau, 1995). Generally, the biosynthesis of terpenoids is compartmentalized, where the mevalonate pathway is present in the cytosol and the MEP pathway in the plastids (Aharoni et al., 2005; Croteau et al., 2000). Generally, sesquiterpenes, triterpenes and polyterpenes are syntheised in the cytosol, whilst monoterpenes, diterpenes and tetraterpenes are largely synthesised in plastids (Croteau et al., 2000).

2.10.2.1 Mevalonate (mevalonic acid) and MEP pathways of terpenoid biosynthesis

Both mevalonate and MEP pathways are initiated by pyruvate (Fig. 2.20) which is produced during the process of photosynthesis, followed by a series of steps which ultimately synthesise different types of terpenoids. In mevalonate pathway, pyruvate is converted to acetyl- CoA, acetoacetyl CoA and 3- hydroxyl-3-methyl-glutanyl-

CoA (HMG- CoA) which is then converted to mevalonic acid (Fig. 2.21). From mevalonic acid, isopentenyl phosphate (IPP) (Fig. 2.22) is derived which is subsequently converted to different types of terpenoids (Fig. 2.24). However, in MEP pathway, pyruvate is converted to glyceraldehydes 3-phosphate (GAP) and 1-deoxy-D-xylulose which produces 2-C-methyl-D-erythritol-4-phosphte (Fig. 2. 23), which is later converted to IPP. Then the major sub groups of terpenoids are synthesised from IPP (Croteau et al., 2000).

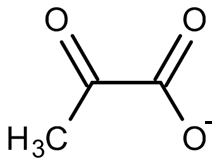


Fig. 2.20 Pyruvate

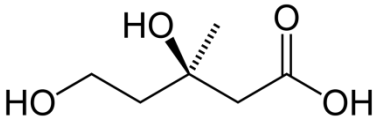


Fig. 2.21 Mevalonic acid

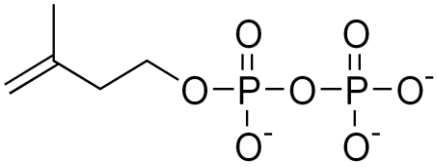


Fig. 2.22 Isopentenyl phosphate (IPP)

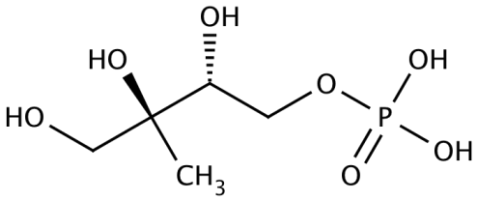


Fig. 2.23 2-C-Methyl-D-erythritol-4-Phosphate

(Source: https://www.sigmaaldrich.com)

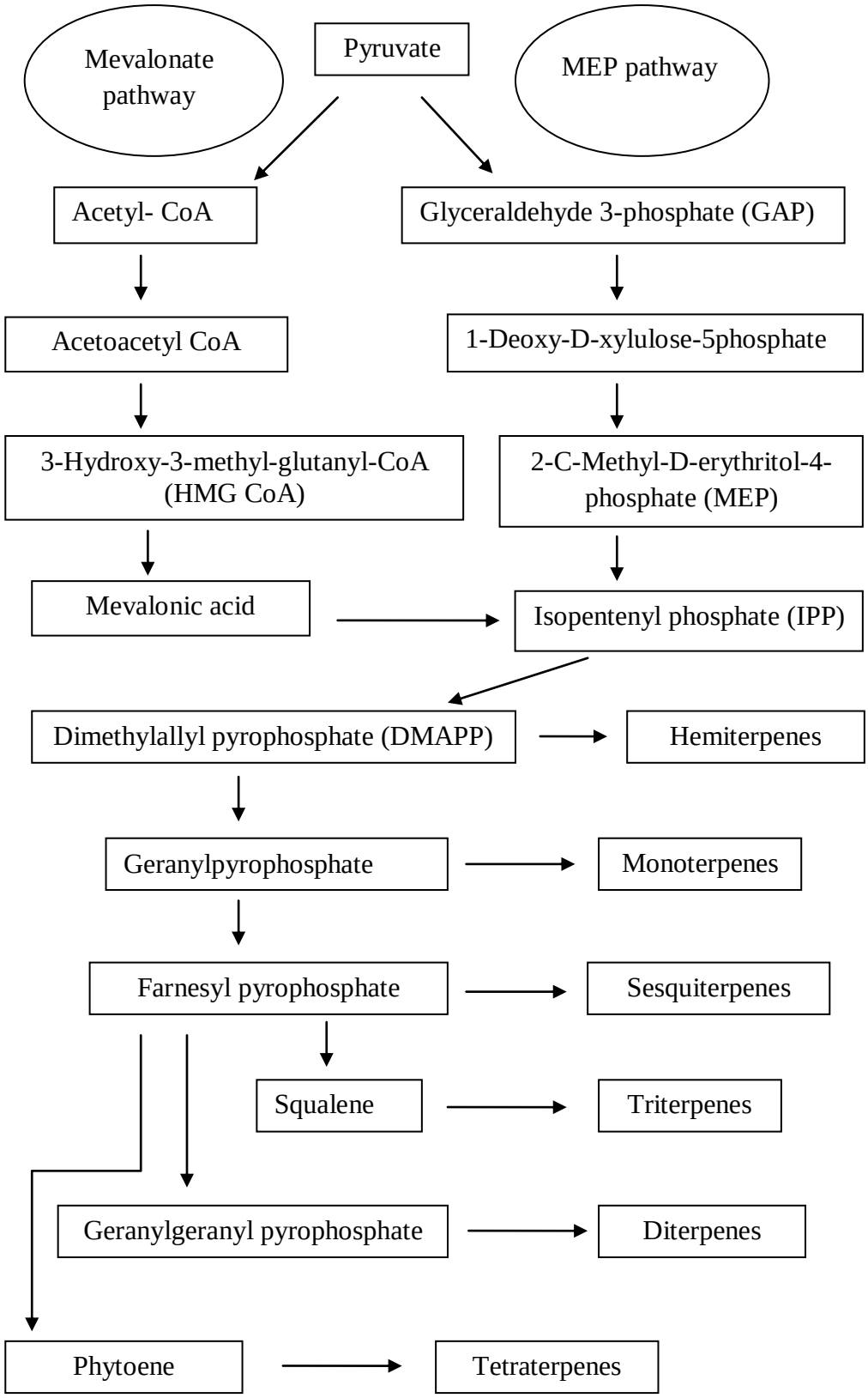


Fig. 2.24 Major steps of Mevalonate and MEP pathways of terpenoid biosynthesis modified from Croteau et al. (2000)

2.11 Tools to increase the concentration of health-promoting compounds in mango fruit

Due to the increasing awareness of the health benefits of secondary compounds produced in plants, the concern of consumers has been shifted from the external quality of fruit and vegetables to their concentration of health-promoting compounds (Schreiner and Huyskens-Keil, 2006). Thus, a need of new technologies to add value to fruit and vegetables by increasing their concentration of desired health-promoting compounds has been created (Cisneros-Zevallos, 2003). In addition to their health prospects, these value added commodities would improve trade prospects of growers and food industry in growing health-oriented markets.

2.11.1 Genetic engineering

Genetic engineering is commonly used to produce crops with higher concentrations of desirable bioactive compounds (Flinn and Zavon, 2004). Biosynthetic pathways of secondary metabolites have been genetically enhanced by the over-expression of regulatory genes that encode different enzymes or regulatory proteins (Verpoorte et al., 2000; Verpoorte and Memelink, 2002). Due to the high antioxidant potential of flavonoids, their biosynthesis in food crops has been often targeted using genetic engineering. Chalcone isomerise (CHI) is one of the enzymes found to be responsible for the synthesis of flavonoids. The over-expression of *Petunia* CHI gene could lead to a 78-fold and a 21-fold increase in the concentration of flavonoids in the peel and paste of tomato respectively (Muir et al., 2001). Similarly, over-expression of *S*-linalool synthase transgene could increase the concentration of monoterpenoid flavour compound *S*-linalool in tomato fruit by many folds (Lewinsohn et al., 2001). Moreover, over-expression of a specific bacterial gene could lead to a 50-fold increase in the level of *β*-carotene in canola seeds (Shewmaker et al., 1999), whilst it was increased by 3-fold in tomato by the over-expression of bacterial phytoene desaturase enzyme in plastids (Romer et al., 2000). Anthocyanins are an important group of flavonoids that determine the colour of many fruit (Espley et al., 2007). Therefore; genetic manipulation of these compounds are also being attempted. Regulation of anthocyanin biosynthesis in apple fruit was found to be controlled at the level of transcription. *MdMYB1*, *MdMYB10* and *MdMYBA* transcription factors

were found to act as activators of anthocyanin biosynthesis (Ban et al., 2007; Espley et al., 2007).

However, an extensive knowledge on the biosynthetic pathways of secondary compounds and the genes involved in the production of these compounds are required to explore the full potential of this technique. The incomplete knowledge on the genes involved and metabolic pathways is a major constraint in genetic engineering (Verpoorte et al., 2000). It is also technically complex and in most cases the genetically modified plants are considered as potential threats that create an ecological imbalance (Cisneros-Zevallos, 2003). Moreover, genetically modified food is generally not accepted by consumers. Any attempt to increase the levels of health-promoting compounds in mango fruit through genetic engineering is not reported yet.

2.11.2 Plant cell culture

Plant cell culture is another potential alternative for the production of desired secondary metabolites that are generally difficult to synthesise chemically or extract directly from plants (Mulabagal and Tsay, 2004; Zhao et al., 2005). However, low yield of compounds obtained via this method is one of the key constraints (Zhao et al., 2005). The productivity of many compounds is still not competitive enough for commercial applications (Zhao et al., 2005). The production of shikonin by *Lithospermum erythrorthizon* cell cultures and taxol by *Taxus* cell cultures are the only successfully industrialized commercial applications so far (Zhao et al., 2005) .

2.11.3 Elicitors

Application of physical and chemical elicitors is becoming the most promising alternative to overcome the constraints faced with genetic engineering and plant cell culture (Ruiz-García and Gómez-Plaza, 2013). Low temperature, ultraviolet and gamma irradiation and altered gas composition are among the physical elicitor treatments, whilst plant signalling molecules such as methyl jasmonate (MeJA), salicylic acid (SA) and ethylene are considered as the chemical elicitors (Schreiner and Huyskens-Keil, 2006). Plant defence against biotic or abiotic stress conditions

involves triggered synthesis of low molecular weight compounds called phytoalexins (Gundlach et al., 1992). Thus, a chemical elicitor can be considered as a compound that can stimulate phytoalexin accumulation in plants (Zhao et al., 2005).

Since plant secondary metabolites are generally synthesised to protect plants from various biotic and abiotic stresses including pest and disease attacks, moisture stress and extreme temperatures; stress induction could be used to stimulate the production of such plant secondary metabolites (Zhao et al., 2005). There is growing evidence on the ability of plants to biosynthesize higher concentrations of secondary metabolites as a response to induced abiotic stresses (Jacobo-Velázquez and Cisneros-Zevallos, 2012). Organically grown fruit are believed to contain more secondary metabolites as such situations induce plants and fruit to use their own natural defence mechanisms against biotic and abiotic stress (Ribeiro et al., 2008). Moreover, significantly higher levels of phenolic acids and proanthocyanidins were recorded in 'Caldesi 2000' nectarine fruit grown on trees subjected to regulated deficit irrigation (RDI) with only 75% of the water applied as compared to the control trees (Pliakoni et al., 2010). Similarly, RDI at two levels; 82% reduction (severe) and 69% reduction (moderate) compared to the estimated crop water requirement could significantly increase the concentrations of antioxidants and ascorbic acid in 'Flordastar' early peaches compared to the control (Falagán et al., 2016).

Hence, targeted postharvest elicitor treatments could be used as a promising tool to produce fruit and vegetables with higher concentrations of phytochemicals to cater the growing consumer demand (Schreiner and Huyskens-Keil, 2006). This technique is considered as a promising practical, effective and safe tool when compared with genetic engineering and plant cell culture (Cisneros-Zevallos, 2003; Ruiz-García and Gómez-Plaza, 2013). The role of application of different physical and chemical elicitors in regulation of biosynthesis of health promoting compounds in mango fruit is yet to be investigated in detail.

2.11.4 Stimulation of terpenoid biosynthesis

The stimulation of terpenoid biosynthesis would possibly increase the concentrations of health-promoting compounds such as carotenoids and lupeol in mango fruit. As mentioned under the section 2.11.2.1, the conversion of acetyl-CoA to acetoacetyl-CoA and 3-hydroxy-3-methylglutaryl-CoA (HMG- CoA) are considered as the first two steps in terpenoid biosynthesis via mevalonic acid pathway (McGarvey and Croteau, 1995). The enzymes that catalyse these two reactions utilize bivalent metal ion Fe^{2+} as a cofactor (McGarvey and Croteau, 1995). Subsequently, isopentenyl pyrophosphate (IPP), geranyl pyrophosphate (GPP) and farnesyl pyrophosphate which involve in the production of hemiterpenes, monoterpenes, sesquiterpenes, diterpenes and tetraterpenes are synthesized. Mg^{2+} or Mn^{2+} is utilized as cofactors by the enzymes involved in the synthesis of these compounds (Fischbach et al., 2000; McGarvey and Croteau, 1995). The addition of Mn^{2+} as bivalent metal ion cofactor resulted in higher activity of monoterpene synthase enzyme isolated from lemon fruit (Lucker et al., 2002), whilst the addition of Mg^{2+} induced *in vitro* terpenoid biosynthesis in tomato cell cultures (Rohdich et al., 2000). The activity of monoterpene synthase enzyme was stimulated by the addition of Mg^{2+} and Mn^{2+} in the needles of Norway spruce {*Picea abies* (L.) Karst.} and by the presence of Mg^{2+} in holm oak leaves (*Quercus ilex* L.) (Fischbach et al., 2000). However, the effect of exogenously applied nutrients acting as enzyme cofactors such as Mg^{2+}, Mn^{2+} and Fe^{2+} on the induction of terpenoid biosynthesis in mango fruit is still unknown. As increased terpenoid biosynthesis would increase the levels of health-beneficial terpenes such as lupeol and carotenoids in mango fruit, the effect of application of above mentioned nutrients on these compounds warrants to be investigated.

2.12 Conclusion

Overall, mango fruit is a rich source of many health-beneficial phytochemicals. However, there is much scope for increasing the levels of these compounds and treatments through correct manipulation of different pre- and postharvest conditions that affect their biosynthesis and maintenance in ripe mango fruit.

CHAPTER 3

General materials and methods

3.1 Fruit and conditions of experiments

Freshly harvested 'Kensington Pride' mango fruit at various maturity stages such as green mature, sprung stage, half ripe and tree ripe from 15- 20 yr old trees grafted on open pollinated unknown mango seedlings were sourced from commercial orchards in Chittering (31° 45′S, 116° 1′E) and Gingin (31° 27′S, 115° 55′E), Western Australia depending upon the requirements of the experiments (Fig. 3.1). The mangoes were washed with chlorinated water and dried before transporting to the Horticulture Research Laboratory, Curtin University, Perth, Western Australia within 2 h. The fruit of uniform size, free from any form of observable deformities, damages or symptoms of disease(s) were selected and treated with the fungicide, Sportak (0.55 ml L^{-1}) containing prochloraz as the active ingredient (Bayer CropScience Pty Ltd., Victoria, Australia). Air dried fruit were packed into soft board trays and transported in a vehicle fitted with air condition to the Horticulture Research Laboratory, Curtin University, Perth, Western Australia and either stored at ambient temperature (21 ± 1.5 °C) until they reach eating soft ripe stage (> 75% yellow skin) or cold stored (5 °C or 13 °C) for 12 or 24 d prior to ripening at ambient conditions. The relative humidity in the cold rooms was maintained at 85% throughout the storage period. The fruit were kept in dark in the cold rooms. Freshly harvested green mature 'R2E2' fruit were obtained from a commercial grower at Dongara (29° 25′S, 114° 93′E), Western Australia and transported to the laboratory in a refrigerated truck on the same day. Selection of fruit and all the other practices were carried out in the same way as described for 'Kensington Pride' mango fruit. The fruit were allowed to ripen at ambient temperature (21 ± 1.5 °C) until eating soft ripe stage (> 75% yellow skin) (Fig. 3.2).

3.2 Sample preparation

Pulp and peel samples were carefully separated in all the experiments for the biochemical analyses. Separation of peel from the pulp was done using a sharp knife.

Pulp cut in to small cubes (0.5 – 1.0 cm^3) and peel ut into small pieces were immediately stored at -80 °C for later analysis of the health-promoting compounds unless otherwise used directly after sample preparation. Freeze dried at – 50 °C and 1×10^{-1} mB (Telstar Cryodos V 1.0, Terrassa, Spain) and powdered samples of pulp and peel were used to analyze lupeol, mangiferin and phenolic acids (gallic acid, chlorogenic acid, vanillic acid, ferulic acid, caffeic acid and/ or quercetin). All the other compounds (total phenols, total antioxidants, ascorbic acid and carotenoids) were analyzed using either fresh or thawed pulp and/or peel.

Green mature | Sprung stage

Half ripe | Tree ripe

Fig. 3.1 'Kensington Pride' Mango fruit harvested at various fruit maturity stages; green mature (light cream pulp, green peel, hard), sprung stage (cream pulp, green peel, springy), half ripe (yellow pulp, 50-60% yellow skin, slightly soft) and tree ripe (yellowish orange pulp, >75% yellow skin, eating soft) (Lalel et al., 2003a)

Chemical compounds studied in this thesis:

Lupeol (PubChem CID: 259846); mangiferin (PubChem CID: 5281647); gallic acid (PubChem CID: 370); chlorogenic acid (PubChem CID: 1794427); vanillic acid

(PubChem CID: 8468); ferulic acid (PubChem CID: 445858); caffeic acid (PubChem CID: 689043); ascorbic acid (PubChem CID: 54670067).

3.3 Chemicals

All reagents and standards of phenolic acids (chlorogenic acid, vanillic acid, gallic acid, quercetin, caffeic acid and ferulic acid), lupeol, mangiferin, β-carotene and ascorbic acid, $MnSO_4$, methyl jasmonate and salicylic acid were purchased from Sigma Aldrich (St. Louis, MO, USA) whilst methanol, acetonitrile and n-hexane were purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Taren Point, NSW, Australia). $FeSO_4$ was purchased from Ajax Finechem (Auburn, NSW, Australia) and $MgSO_4$ was purchased from VWR International Pty Ltd. (Poole, England). Nitric oxide in Nitrogen was purchased from BOC Australia, Sydney, NSW, Australia. All the reagents and standards used were of HPLC grade.

Fig. 3.2 ‘R2E2’ mango fruit at eating soft stage

3.4 The layout of different experiments

The experiments were carried out following one or two factor factorial completely randomized design (CRD) or randomized block design (RBD). Number of fruit per replication was 10 for every experiment and replicated either 3 or 4 times.

3.5 Determination of health-promoting compounds in the pulp and peel of ripe mango fruit

3.5.1 Lupeol

Extraction of lupeol

The method described by Ruiz-Montanez et al. (2014) was used for the extraction of lupeol with slight modifications. For this extraction, 10.0 g of freeze dried pulp/peel powder was taken in a 250.0 ml Schott bottle and 100.0 ml of n-hexane (100%) was added to it as the extraction solvent. The sample: solvent ratio of 1:10 (g sample: ml solvent) was maintained in extraction of all the samples. The mixture was homogenized for 30 s (DIAX900, Heidolph Co., Ltd., Schwabach, Germany) and then sonicated (Soniclean Pvt. Ltd., Therbaton, South Australia, Australia) for 30 min at a constant frequency of 42 kHz. The sonicated samples were centrifuged at 5,000 × g for 20 min at 4 °C (Eppendorf centrifuge 5810 R, Hamburg, Germany). The supernatant was filtered through Whatman number 1 filter paper. The filtrate was dried using a (BÜCHI Rotavapor® R 205 equipped with BÜCHI heating bath B 490 and BÜCHI vacuum controller V 800, BÜCHI Labortechnik AG, Flawil, Switzerland). The residue was dissolved in 1.0 mL of acetonitrile and stored at -20 °C until analysis within a week. The extraction method of lupeol is summarized below (Fig. 3.3).

The identification and quantification of lupeol in mango pulp/peel was carried out following the method described by Oliveira et al. (2012) with some modifications, using an Agilent HPLC system (Agilent Technologies, 1200 series, Ratingen, Germany) fitted with a diode array detector (DAD) (Agilent Technologies, 1200 Infinity, Ratingen, Germany). Absorbance was recorded at 210 nm wavelength and the retention time was 9.13 min. A reversed phase C18 column (4.6 mm×150 mm, 5 µm spherical particle size) was used to separate the peaks. A mobile phase of 99.99% acetonitrile and 0.01% acetic acid at a flow rate of 1.2 mL min^{-1} was used for the determination of lupeol. The total run time was 30 min including the column wash. The injection volume of the sample was 20.0 µL. Lupeol was identified based on the retention time of the standard (Fig. 3.4 A) and confirmed by spiking (Fig. 3.4 B).

Lupeol was quantified using a lupeol standard curve ($y = 160.03\ x$, $R^2 = 0.9893$). The concentration of lupeol in mango samples (Fig. 3.4 B) was expressed as mg kg^{-1} dry weight basis.

The standard curve was drawn using the absorption at 5.0 mg L^{-1}, 10.0 mg L^{-1}, 20.0 mg L^{-1}, 50.0 mg L^{-1} and 100.0 mg L^{-1}. The standard solutions were prepared using a 1000.0 mg L^{-1} stock solution of lupeol made by dissolving 10.0 mg of lupeol standard in 10.0 mL of HPLC grade acetonitrile.

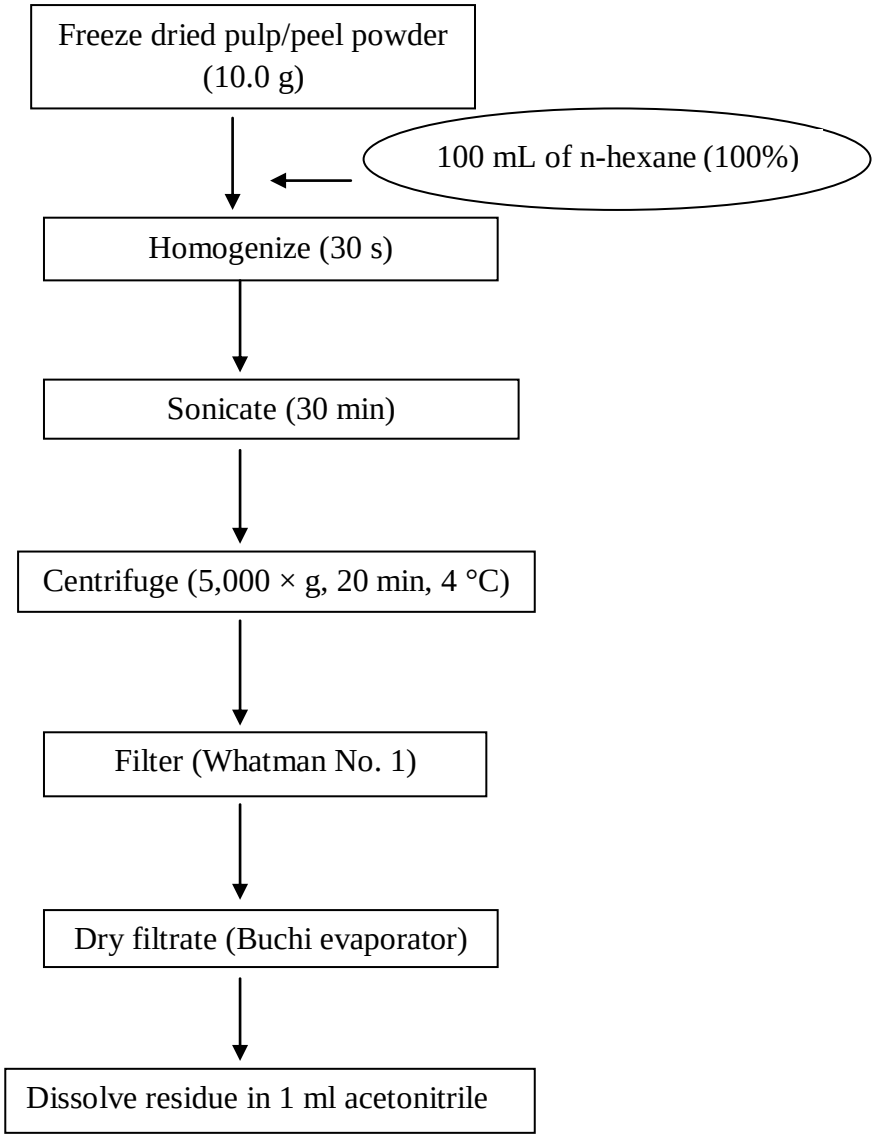


Fig. 3.3 The summary of the method of extracting lupeol from mango pulp/ peel samples

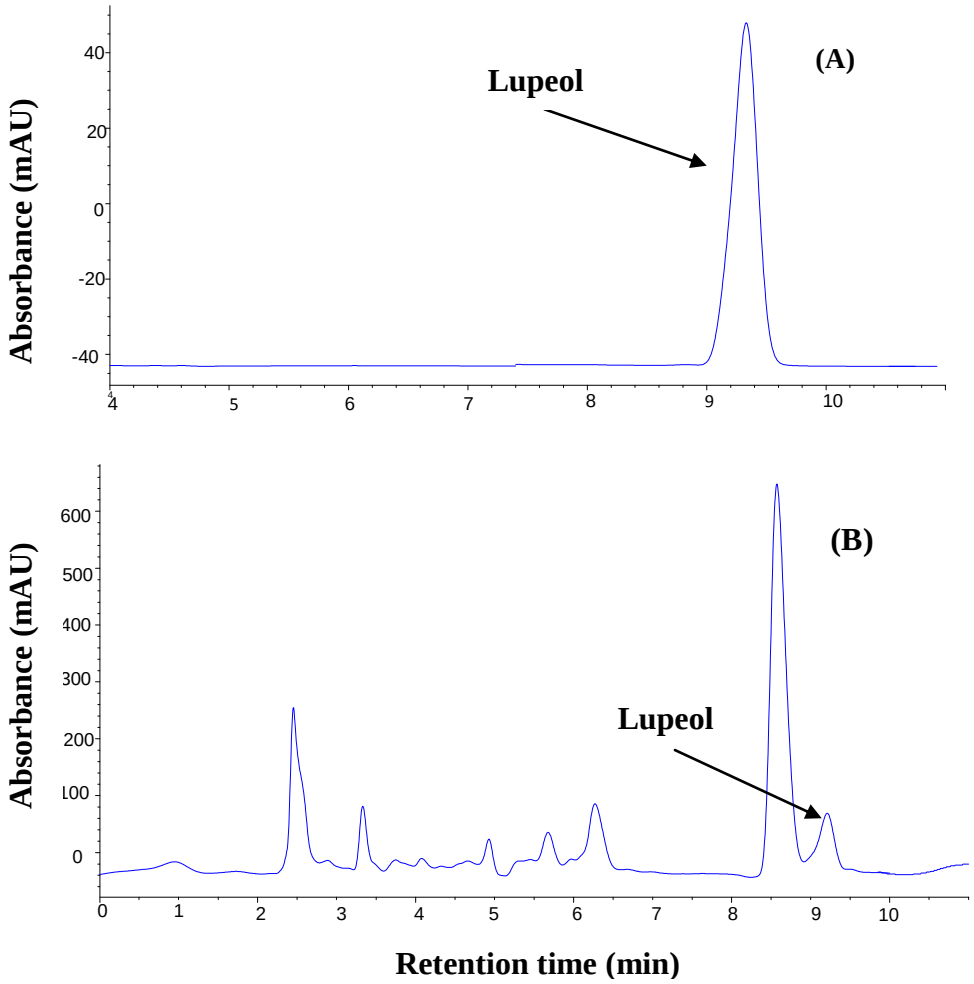


Fig. 3.4 Lupeol standard (5.0 mg L^{-1}- A), mango pulp sample ('Kensington Pride'- B)

3.5.2 Mangiferin and phenolic acids

Extraction of mangiferin and phenolic acids

The concentrations of mangiferin and different phenolic acids in the pulp and peel of mango fruit were determined using the method described by Palafox-Carlos et al. (2012a) with some modifications (Fig. 3.7). For the extraction of these compounds, 10.0g of freeze dried (Telstar Cryodos V 1.0, Terrassa, Spain) pulp/peel samples were mixed with 100.0 mL 80% methanol (v/v). The mixture was then homogenized for 30 s (DIAX900, Heidolph Co., Ltd., Schwabach, Germany). Homogenization of

the mango sample was followed by sonication for 30 min at a constant frequency of 42 kHz (Soniclean Pvt. Ltd., Therbaton, South Australia, Australia). Following sonication, the sample was centrifuged at 10,000 × g for 25 min at 4 °C (Eppendorf centrifuge 5810 R, Hamburg, Germany) and filtered through Whatman No.1 filter paper. The filtrate was dried using a Buchi rotary evaporator (BÜCHI rotavapor® R 205 equipped with BÜCHI heating bath B 490 and BÜCHI vacuum controller V 800, BÜCHI Labortechnik AG, Flawil, Switzerland). Then the residue was dissolved in 1.0 mL of methanol and stored at -20 °C until analysis within 1 – 2 weeks.

The identification and quantification of the polyphenol profile of mango pulp/peel was carried out using an Agilent HPLC system (Agilent Technologies, 1200 series, Ratingen, Germany) equipped with a diode array detector (DAD) (Agilent Technologies, 1200 Infinity, Ratingen, Germany). Absorbance was recorded at a range of wavelengths (Table 3.1). A reversed phase C18 column (4.6 mm×250 mm, 5 µm spherical particle size) was used for peak separation and 1% formic acid (A) and 99% acetonitrile (B) was used as the mobile phase. The elution gradient was 2-100% (B) in 40 min at a flow rate of 0.5 mL min^{-1}. The total run time was 60 min including column wash. The injection volume was 20.0 µL. Mangiferin and phenolic acids were identified using the retention times of standards and confirmed by spiking (Fig. 3.5). The concentrations of individual polyphenols (Fig. 3.6) were quantified using the standard curves of gallic acid (y = 106.98 x, R^2 = 0.9999), chlorogenic acid (y = 64.572 x, R^2 = 0.9999), vanillic acid (y = 121.26 x, R^2 = 0.9994), quercetin (y = 47.873 x, R^2 = 0.9989), ferulic acid (y = 126.89 x, R^2 = 1), caffeic acid (y = 227.17 x, R^2 = 0.9849) and mangiferin (y = 88.17 x, R^2 = 0.9812) and expressed as mg kg^{-1} on dry weight basis.

The standard solutions of 10.0 mg L^{-1}, 20.0 mg L^{-1}, 50.0 mg L^{-1}, 100.0 mg L^{-1} and 200.0 mg L^{-1} were prepared using 1000.0 mg L^{-1} stock solutions of mangiferin, gallic acid, chlorogenic acid, vanillic acid, caffeic acid, ferulic acid and quercetin by dissolving 10 mg of each standard in 10.0 mL of HPLC grade methanol.

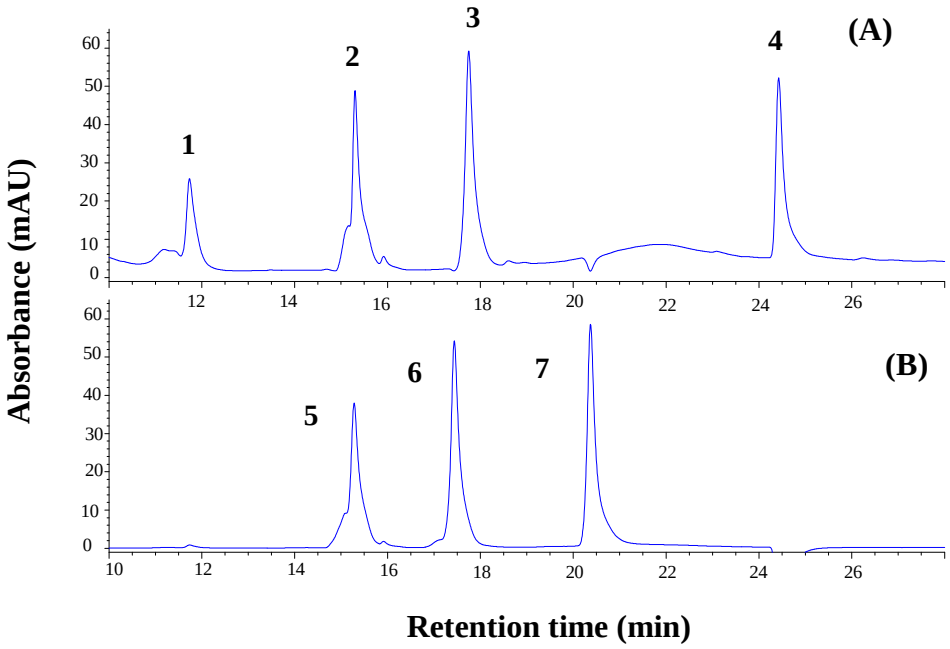


Fig. 3.5 Separation of a mixed standard solution (5 mg L^{-1}) of polyphenols by HPLC-DAD at 255 nm (A) and 325 nm (B). 1- Gallic acid, 2- Mangiferin, 3- Vanillic acid, 4- Quercetin, 5- Chlorogenic acid, 6- Caffeic acid, 7- Ferulic acid

Table 3.1 The absorbance wavelength and retention time of each phenolic standard

Standard	Wavelength (nm)	Retention time (min)
Mangiferin	258	15.30
Gallic acid	280	12.71
Chlorogenic acid	325, 320	15.18
Vanillic acid	255	18.19
Ferulic acid	325, 320	20.74
Caffeic acid	325, 320	17.83
Quercetin	255	24.42

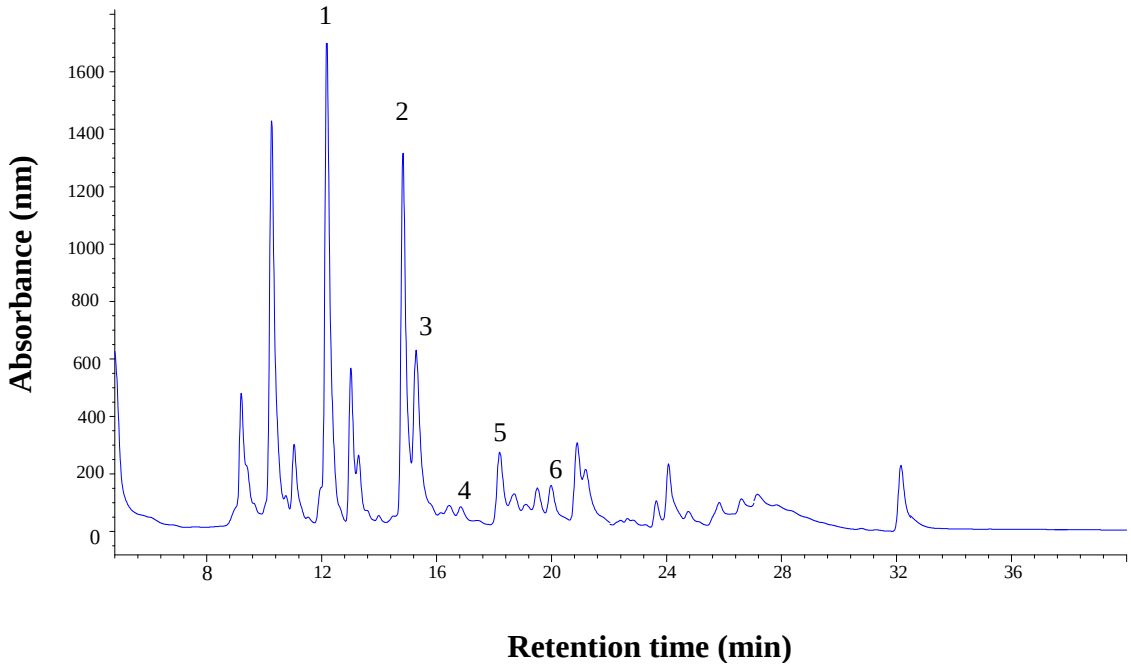


Fig. 3.6 The general polyphenol profile in ripe 'Kensington Pride' mango peel recorded by HPLC-DAD at 280 nm. Peaks 1, 2, 3, 4, 5 and 6 represent gallic acid, chlorogenic acid, mangiferin, caffeic acid, vanillic acid and ferulic acid respectively.

3.5.3 Total phenols

The total phenol concentration of pulp and peel of mango fruit was determined using the method described by Robles-Sànchez et al. (2009) using Folin-Ciocalteu reagent with a few modifications (Fig. 3.8). Representative freshly thawed mango pulp/peel samples (20.0 g) were homogenized for 30 s (DIAX900, Heidolph Co., Ltd., Schwabach, Germany) with 15.0 mL of 80% methanol (v/v). Following the homogenization, the samples were then sonicated for 15 min (Soniclean Pvt. Ltd., Therbaton, South Australia, Australia) at a constant frequency of 42 kHz. The sonicated samples were centrifuged for 15 min at 10,000 × g at 5 °C (Eppendorf centrifuge 5810 R, Hamburg, Germany) and filtered through Whatman No.1 filter paper. The same procedure was followed for the re-extraction of residue one more time. Combined extracts were made to a total of 60.0 mL volume by adding 80%

methanol (v/v). The extract (50.0 µL) was mixed with deionized water (3.0 mL) and two folds diluted Folin-Ciocalteu reagent (250.0 µL) and kept for 5 min in dark. After 5 min, 250.0 µL of a sodium carbonate solution (7%) was added to the above mixture. Finally, the total volume of the solution was made to 5.0 mL by adding deionized water (1450.0 µL). The mixture was then kept in dark for additional 90 min. The absorbance was measured at 750 nm after 90 min using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK). Peel extracts were diluted ten folds. The total phenolic concentration was calculated using a gallic acid standard curve and expressed in g gallic acid equivalents (GAE) kg^{-1} fresh weight basis.

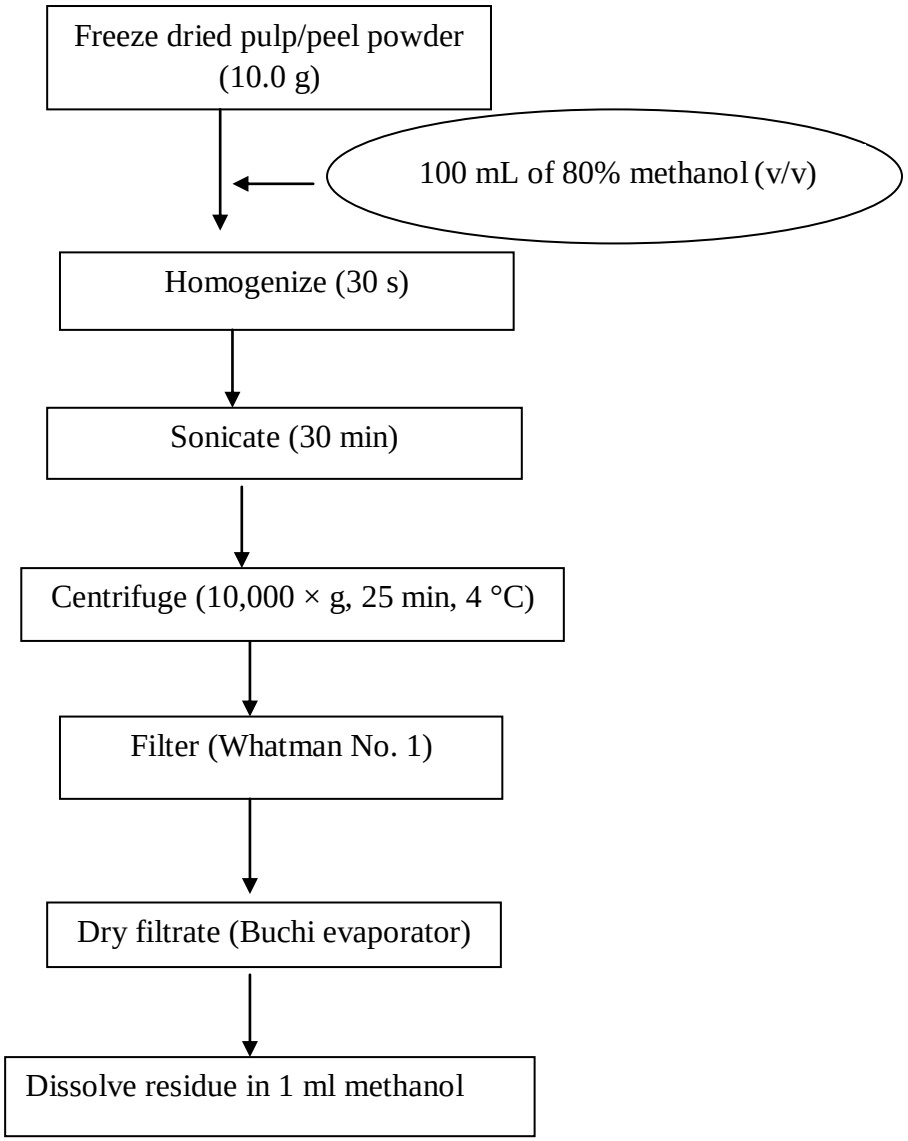


Fig. 3.7 The summary of the method of extracting mangiferin and phenolic acids from mango pulp/ peel samples

3.5.4 Total antioxidant capacity

The total antioxidant capacity of ripe mango pulp/peel was estimated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay described by Brand-Williams et al. (1995) using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK) with slight modifications (Fig. 3.9). Representative pulp/peel samples of mango fruit (5.0 g) were grinded with 200.0 mg of white quartz sand (-50+70 mesh, Sigma Aldrich, USA) with the use of a ceramic mortar and pestle in 10.0 mL of extraction buffer. The extraction buffer was prepared by dissolving 2 mM NaF (84.0 mg L^{-1}) in distilled water (200.0 mL) and then mixing with 800.0 mL of pure methanol. The homogenized sample was centrifuged at 10,000 × g for 20 min at 4 °C (Eppendorf centrifuge 5810 R, Hamburg, Germany). The DPPH stock solution was prepared by dissolving DPPH powder (24.0 mg) in pure methanol (100.0 mL). Fourfold (1:4 v/v) diluted stock solutions were used as working solutions. The absorbance of the working solution was adjusted to 1.1 at 515 nm either by adding more stock solution or pure methanol accordingly (if the absorbance was > 1.1, methanol was added, if it was < 1.1, the stock solution was added). Supernatant of the centrifuged sample (50.0 µL) and the working solution (950.0 µL) were mixed and kept in dark for 15 min. After 15 min the absorbance was recorded at 515 nm using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK). The absorbance was maintained between 0.6 and 0.7 by adjusting sample dilutions. Total antioxidant capacity was determined using a standard curve of Trolox (6-hydroxy-2, 5, 7, 8-tetramethychromane-2-carboxylic acid) and was expressed as mmol Trolox equivalent antioxidant capacity (TEAC) kg^{-1} fresh weight basis.

3.5.5 Ascorbic acid

The concentration of ascorbic acid in mango pulp was determined by following the method described earlier by Malik and Singh (2005) (Fig. 3.10). For the quantification of ascorbic acid, representative pulp sample (5.0g) was grinded with 200 mg of white quartz sand (-50+70 mesh, Sigma Aldrich, USA) and 20.0 mL of 6% metaphosphoric acid with ethylenediaminetetraacetate acid disodium salt

(EDTA) (0.18 g). Following homogenization, the mixture was centrifuged at 5,000 × g for 20 min at 4 °C (Eppendorf centrifuge 5810 R, Hamburg, Germany). After centrifugation, the supernatant (400.0 μL) was mixed with 3% metaphosphoric acid (200.0 μL), distilled water (1.4 mL) and five-fold diluted Folin reagent (Folin: distilled water 1:5 v/v) (200.0 μL). The absorbance was recorded after 10 min at 760 nm wavelength using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK). The ascorbic acid concentration was calculated using a standard curve of L-ascorbic acid and was expressed as mg kg^{-1} fresh weight basis.

3.5.6 Total carotenoids

The method described previously by Lalel et al. (2003a) was followed to determine the concentration of total carotenoids in mango pulp with some modifications (Fig. 3.11). Mango pulp (2.0 g) was grinded with magnesium carbonate (50.0 mg) and the mixture was homogenized with 20 mL of acetone: n-hexane (75:60, v/v) extraction solution (DIAX900, Heidolph Co., Ltd., Schwabach, Germany). After centrifuging at 5,000× g for 20 min at 4 °C the supernatant was collected and the extraction process was repeated once for the residue to ensure maximum extraction. Then the combined supernatant was washed with 40.0 ml of 10% Sodium chloride by shaking (Ratek Orbital mixer, Ratek Instruments Pty, Ltd., Victoria, Australia) for 5 min. After shaking for 5 min, the bottom layer was discarded and the top layer was separated and shaken twice with 40.0 mL of distilled water to remove acetone. Glass cuvettes were filled with the hexane extract and the absorbance was measured at 436 nm using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essesx, UK). The whole extraction procedure was carried out under subdued light conditions and all glassware used for the extraction was covered with aluminium foil to prevent photo degradation of carotenoids. The concentration of total carotenoids was expressed as mg kg^{-1} fresh weight basis.

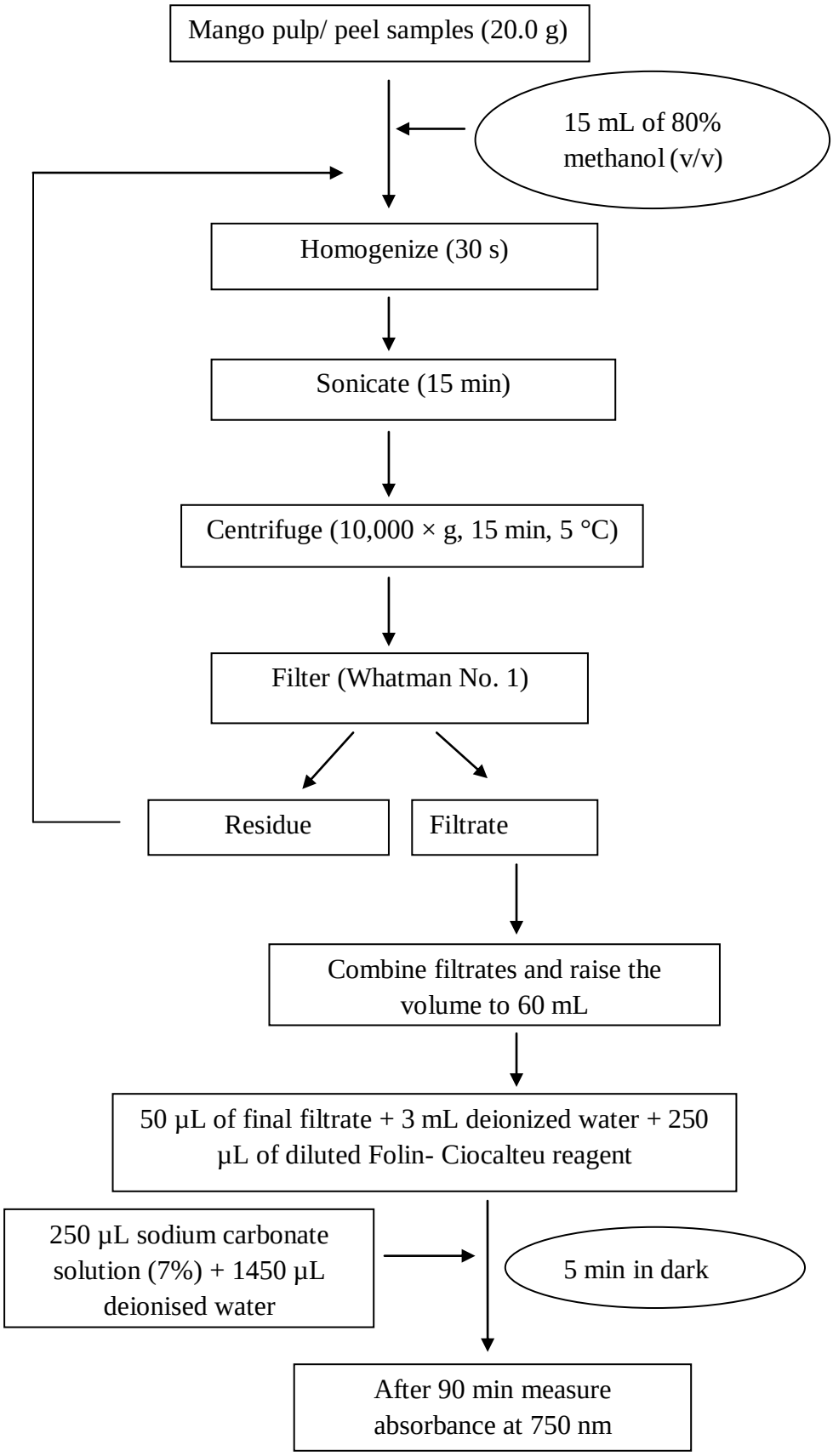


Fig. 3.8 The summary of the method of extraction and determination of total phenols in mango pulp/peel

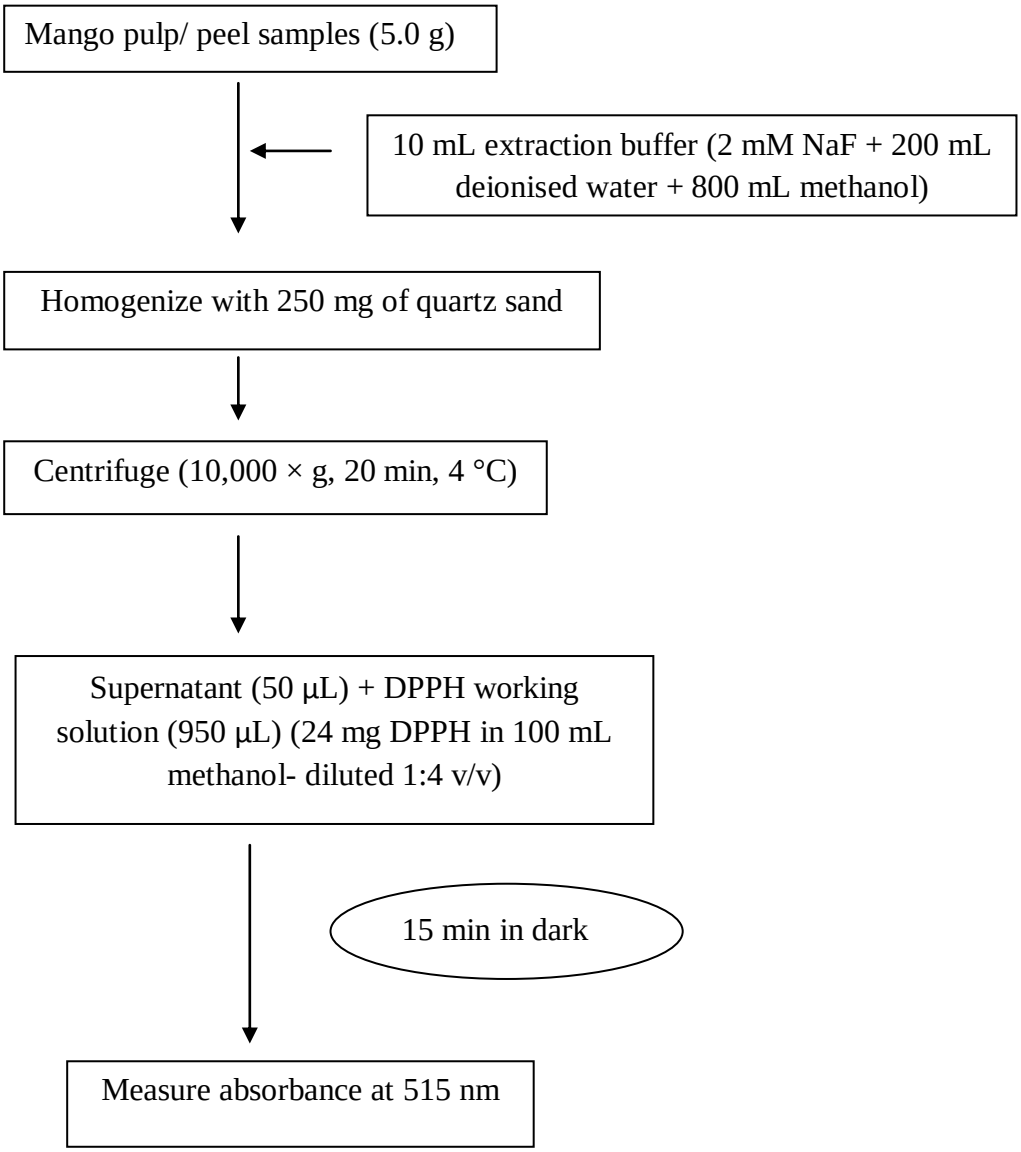


Fig. 3.9 The summary of the method of extraction and estimation of total antioxidant capacity in mango pulp/peel

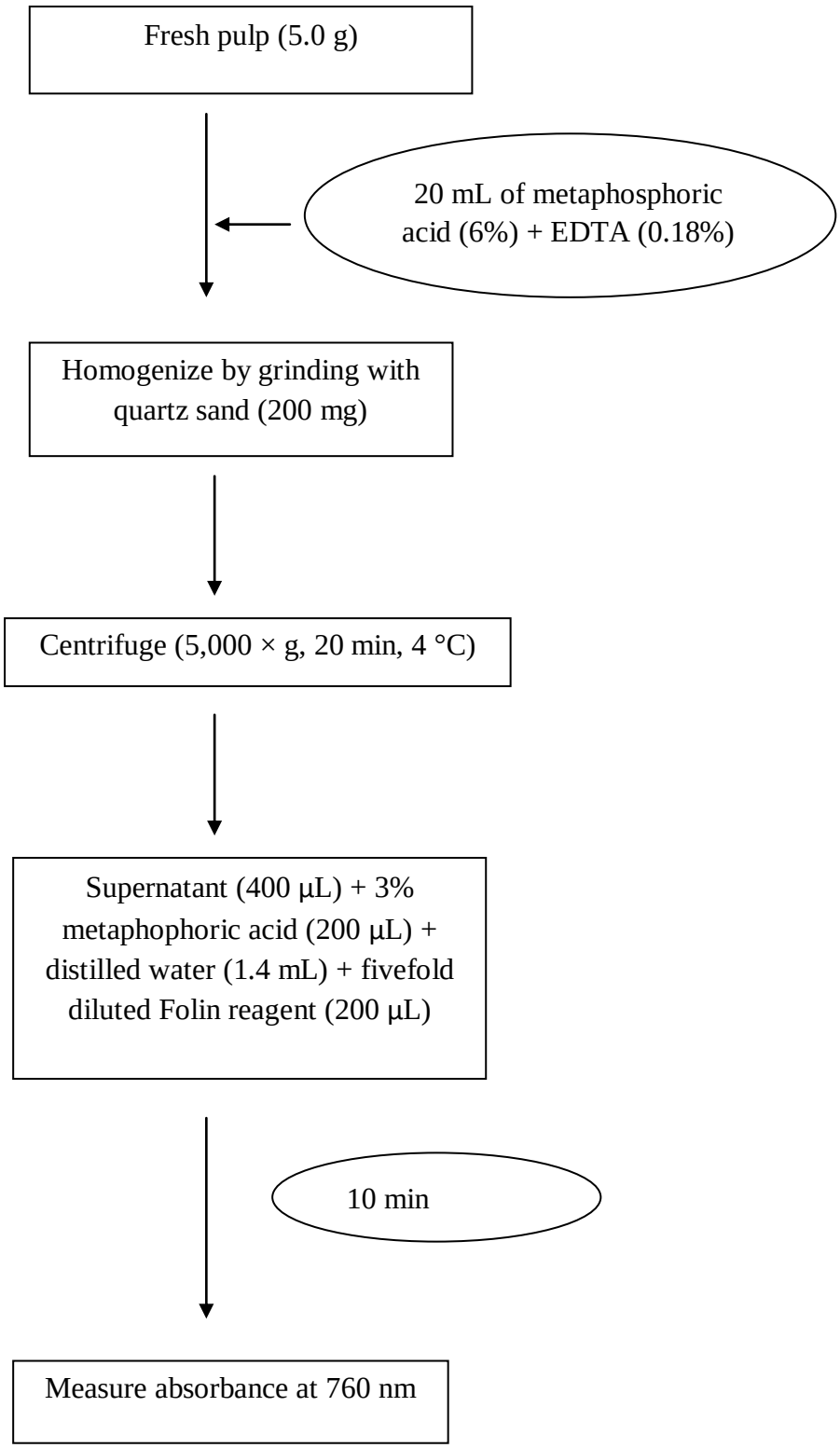


Fig. 3.10 The summary of the method of the determination of ascorbic acid in mango pulp

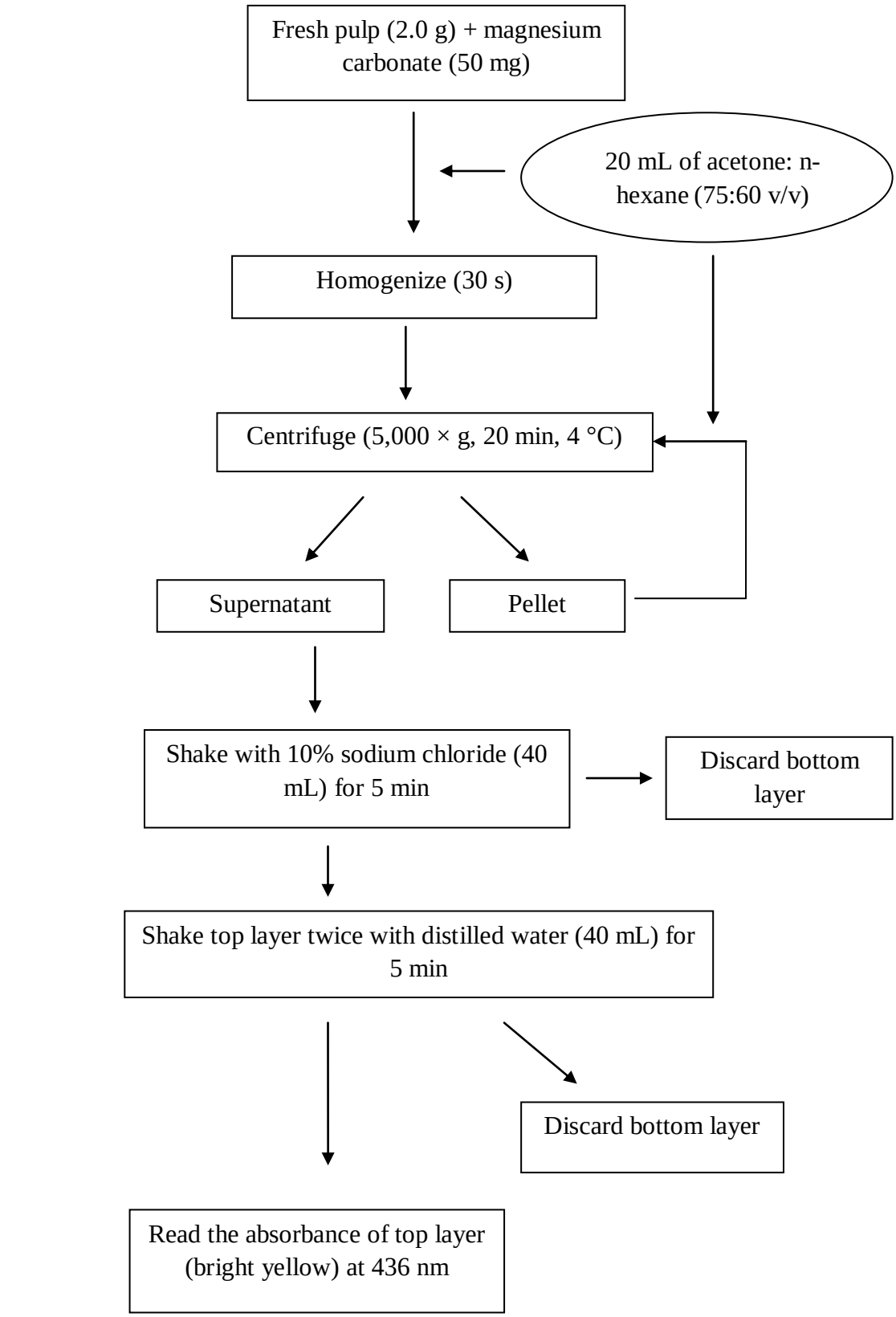


Fig. 3.11 The summary of the method of extraction and determination of total carotenoids in mango pulp

3.6 Determination of the rate of ethylene production during mango fruit ripening

The method explained by Bulens et al. (2014) was followed to determine the rate of ethylene production using a laser-based photoacoustic ethylene detector (ETD-300, Sensor Sense, Nijmegen, The Netherlands) with some modifications. The ethylene production of six mango fruit sealed in glass jars at room temperature and atmospheric pressure was measured in a continuous cycle of 20 min each (Fig. 3.12A and 3.12B). The stable ethylene reading was obtained after 10 – 12 min (Fig. 3.13). Carbon dioxide and water scrubbers (soda lime and calcium chloride respectively) were fixed to remove these compounds from gas samples before analysis (3.14). Two fruit from each replication were used to record the daily rate of ethylene production from the date of harvesting until the fruit turn eating soft stage.The rate of ethylene production was expressed in pmol kg^{-1} s^{-1}.

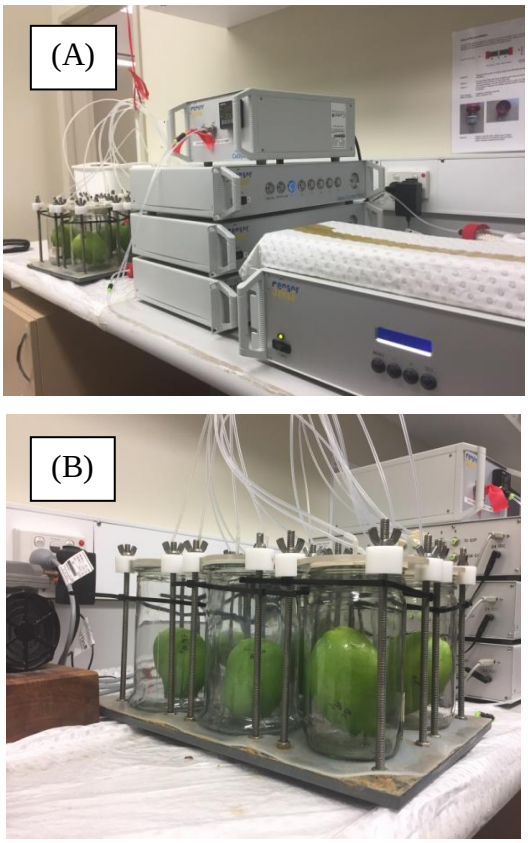


Fig. 3.12 Determination of the rate of ethylene production in mango fruit (A) sealed in glass jars (B)

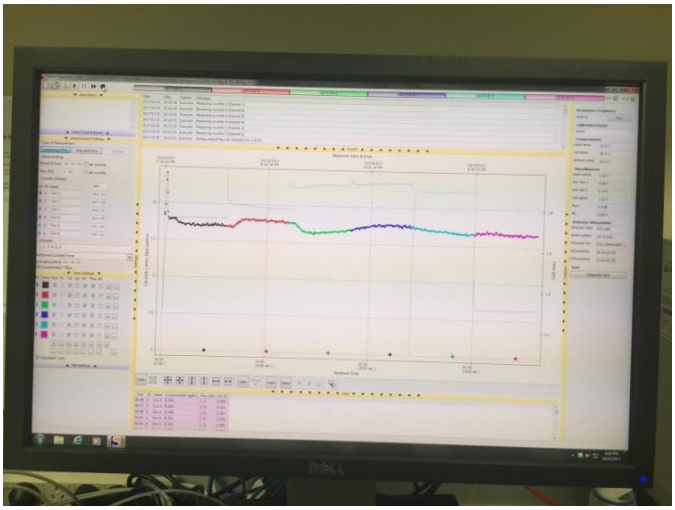


Fig. 3.13 The stable ethylene reading recorded by laser-based photoacoustic ethylene detector

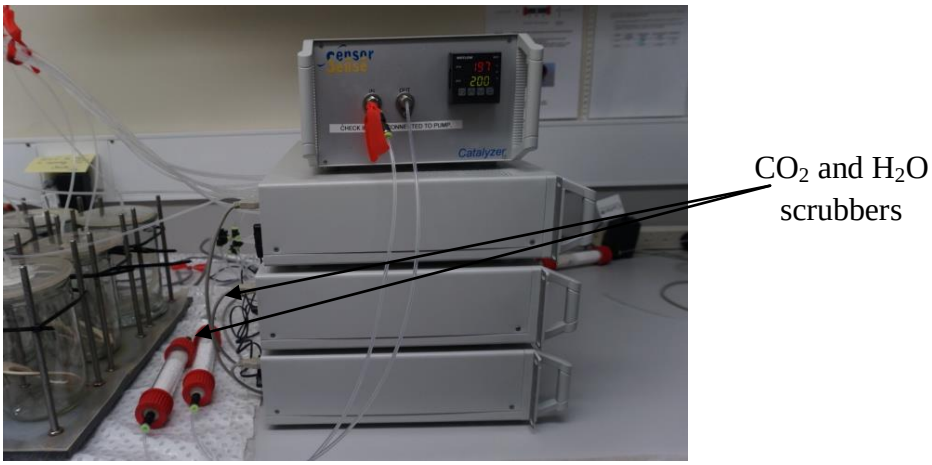


Fig. 3.14 Carbon dioxide and water scrubbers

3.7 Estimation of the rate of respiration during mango fruit ripening

The method previously described by Zaharah and Singh (2011a) was followed to estimate the rate of respiration. An infrared gas analyzer (Servomex Gas Analyzer, series 1400, East Sussex, UK) with Powerchrome 2 software was used to determine the amount of carbon dioxide produced during respiration. Four fruit of each replication were used for the estimation of respiration rate. Fruit were sealed in separate glass jars (1.0 L) with a rubber septum fixed on the lid for 1 h at room temperature (21±1 °C). After 1 h, the headspace gas (2.0 mL) was injected to the

infrared gas analyzer following 2 consecutive injections (2.0 mL each) of carbon dioxide standard (8.3±0.16% in N_2). The sample area and standard area of the peaks obtained from the infrared gas analyzer (Fig. 3.15) were used to calculate the rate of respiration and the values were expressed in μmol kg^{-1} s^{-1}.

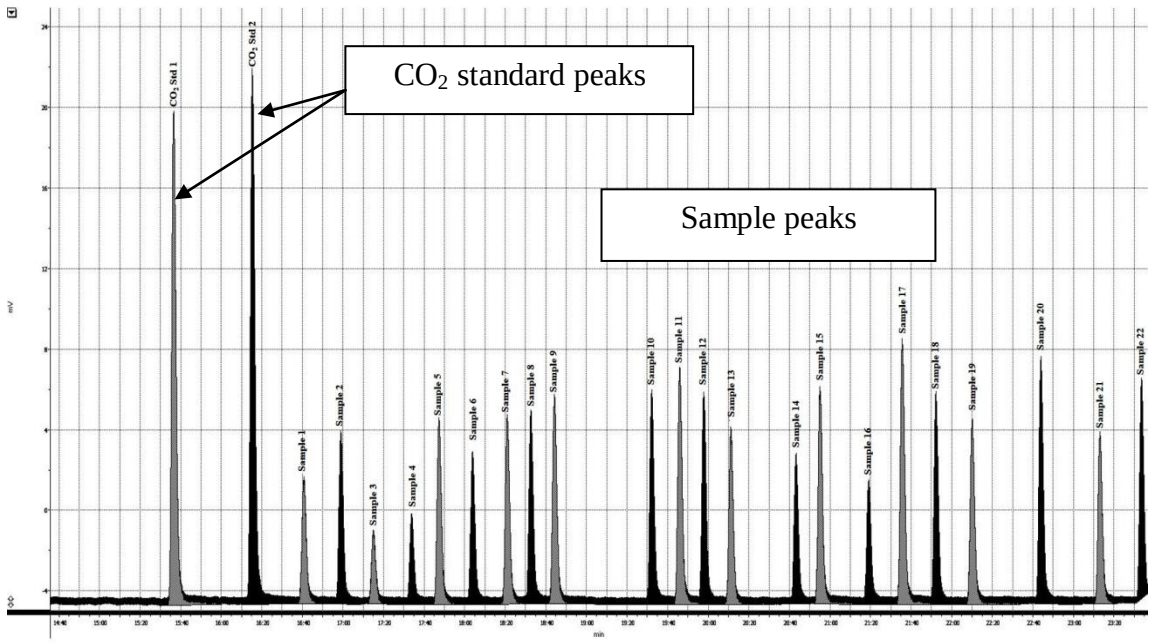


Fig. 3.15 Standard and sample peaks of carbon dioxide obtained from infrared gas analyzer

3.8 Data analysis

Depending upon the experiment, the data was subjected to one way or two-way analysis of variance (ANOVA) using Genstat 14.0 software (Lawes Agricultural Trust, Rothamsted Experimental station, UK). The results were recorded as means ± standard deviation of the mean (SD). Least significant difference (LSD) was calculated following significant ($P \leq 0.05$) F-test. Duncan multiple comparison test or Fisher's protected least significant difference test was used to calculate the significant differences (LSD).

CHAPTER 4

Pre-harvest spray application of $FeSO_4$ (Fe^{2+}), $MgSO_4$ (Mg^{2+}) and $MnSO_4$ (Mn^{2+}) modulates the concentrations of terpenoids, mangiferin and phenolic acids in ripe mango fruit

ABSTRACT

Bivalent metal ions Fe^{2+}, Mg^{2+} and Mn^{2+} are enzyme cofactors in terpenoids biosynthesis. The effects of spray application of aqueous $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.2% or 0.3%) 30 d prior to harvest on the levels of terpenoids (lupeol and total carotenoids), mangiferin, phenolic acids, total phenols, total antioxidants and ascorbic acid in ripe mango fruit were investigated. The concentration of lupeol was significantly higher in the peel with spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.2% and 0.3%) compared to the control. The application of 0.3% of $FeSO_4$, $MgSO_4$ and $MnSO_4$ increased ($P \leq 0.05$) total carotenoids in the pulp. 0.2% $FeSO_4$, $MgSO_4$ and $MnSO_4$ treatments significantly increased the levels of mangiferin in the pulp compared to 0.3% treatments and control. The concentrations of gallic and ferulic acids in the peel and chlorogenic acid in the pulp were highest ($P \leq 0.05$) in the fruit sprayed with 0.2% $FeSO_4$. In conclusion, spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.2% and 0.3%) 30 d before harvest may increase the concentrations of lupeol in the peel, 0.3% of these nutrients may increase the level of carotenoids in the pulp and 0.2% $FeSO_4$ may increase phenolic acids in pulp/peel of ripe mango fruit.

KEYWORDS: *Mangifera indica* L.; terpenoid biosynthesis; Fe^{2+}; Mg^{2+}; Mn^{2+}; health-promoting compounds,

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4.1 Introduction

Terpenoids contribute significantly to the survival of plants by playing a range of physiological roles as plant growth regulators, photosynthetic pigments, and structural components in cell membranes. Additionally, terpenoids also provide humans with a number of health benefits through their high antioxidant potential as well as flavouring agents and fragrances in food and cosmetic industries (Aharoni et al., 2005; McGarvey and Croteau, 1995).

All terpenoids are produced by either the mevalonate pathway (Fig. 4.1), which is active in the cytosol, or by the plasticidal 2-C-methyl-D-erythritol-4-phophate (MEP) pathway (Aharoni et al., 2005; McGarvey and Croteau, 1995). Both primary and secondary metabolites are generated during terpenoid biosynthesis (Aharoni et al., 2005; McGarvey and Croteau, 1995). Carotenoids and chlorophylls are among the primary terpenoid metabolites produced by this pathway, whilst monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}) and triterpenoids (C_{30}) are among the secondary metabolites (Aharoni et al., 2005). The cytoplasmic mevalonate pathway is generally considered to supply the precursors for the production of sesquiterpenes and triterpenes (Fig. 4.1), whilst MEP pathway in the plastids supplies the precursors for the production of isoprene, monoterpenes, diterpenes and tetraterpenes (e.g. carotenoids).

The first two reactions of the terpenoid biosynthesis (Fig. 4.1), i.e. the conversion of acetyl-CoA to acetoacetyl-CoA and 3-hydroxy-3-methylglutaryl-CoA (HMG- CoA) are catalysed by 2 separate enzymes acetyl-CoA acetyltransferase and HMG- CoA synthase in animals and yeast, whilst in plants these 2 reactions are apparently catalysed by a single enzyme (McGarvey and Croteau, 1995). These enzymes utilize the bivalent metal ion Fe^{2+} as a cofactor in these reactions (McGarvey and Croteau, 1995). Followed by these first two steps, isopentenyl pyrophosphate (IPP) and the building blocks of higher order terpenoids, geranyl pyrophosphate (GPP) and farnesyl pyrophospahate are synthesized (Fig. 4.1). The enzymes involved in the synthesis of these compounds require Mg^{2+} or Mn^{2+} as cofactors (Fischbach et al., 2000; McGarvey and Croteau, 1995).

Lupeol {Lup-20(29)-en-3β-ol} the pentacyclic triterpene is one of the key terpenoids with a wide array of significant health related bioactivity (Gallo and Sarachine, 2009). Lupeol has shown a remarkable efficacy in improving the biomarkers of several chronic human diseases (Siddique and Saleem, 2011). Carotenoids are another important group of terpenoids present in plants (McGarvey and Croteau, 1995) which possess antioxidant and anti-carcinogenic properties that can reduce the risk of several chronic diseases (Masibo and He, 2009). Mango fruit (*Mangifera indica* L.) is considered as a good dietary source of both lupeol (Siddique and Saleem, 2011) and carotenoids (Mercadante and Rodriguez-Amaya, 1998).

As the consumer demand is shifting from the emphasis on the external quality to the level of health-promoting compounds in different fruit and vegetables, a need for the development of simple and practical methods that can increase the concentration of these compounds in fresh produce has recently come forward (Schreiner and Huyskens-Keil, 2006). The stimulation of terpenoid biosynthesis could possibly increase the health benefits of fruit and vegetables, enabling the growers, retailers and food processors to cater for the growing demand of health-oriented markets willing to pay a higher price. Lucker et al. (2002) reported that the addition of Mn^{2+} resulted in higher activity of monoterpene synthase enzyme isolated from lemon fruit (*Citrus limon* L.), whereas Rohdich et al. (2000) reported that the activity of 4-Diphosphocytidyl -2-C-methyl-D-erythritol Kinase required in terpenoid biosynthesis was significantly increased by the addition of Mg^{2+} to cell cultures of tomato fruit. Similarly, the activity of monoterpene synthase was stimulated by the *in vitro* addition of Mg^{2+} and Mn^{2+} in the needles of Norway spruce {*Picea abies* (L.) Karst.} and by the presence of Mg^{2+} in holm oak leaves (*Quercus ilex* L.) (Fischbach et al., 2000).

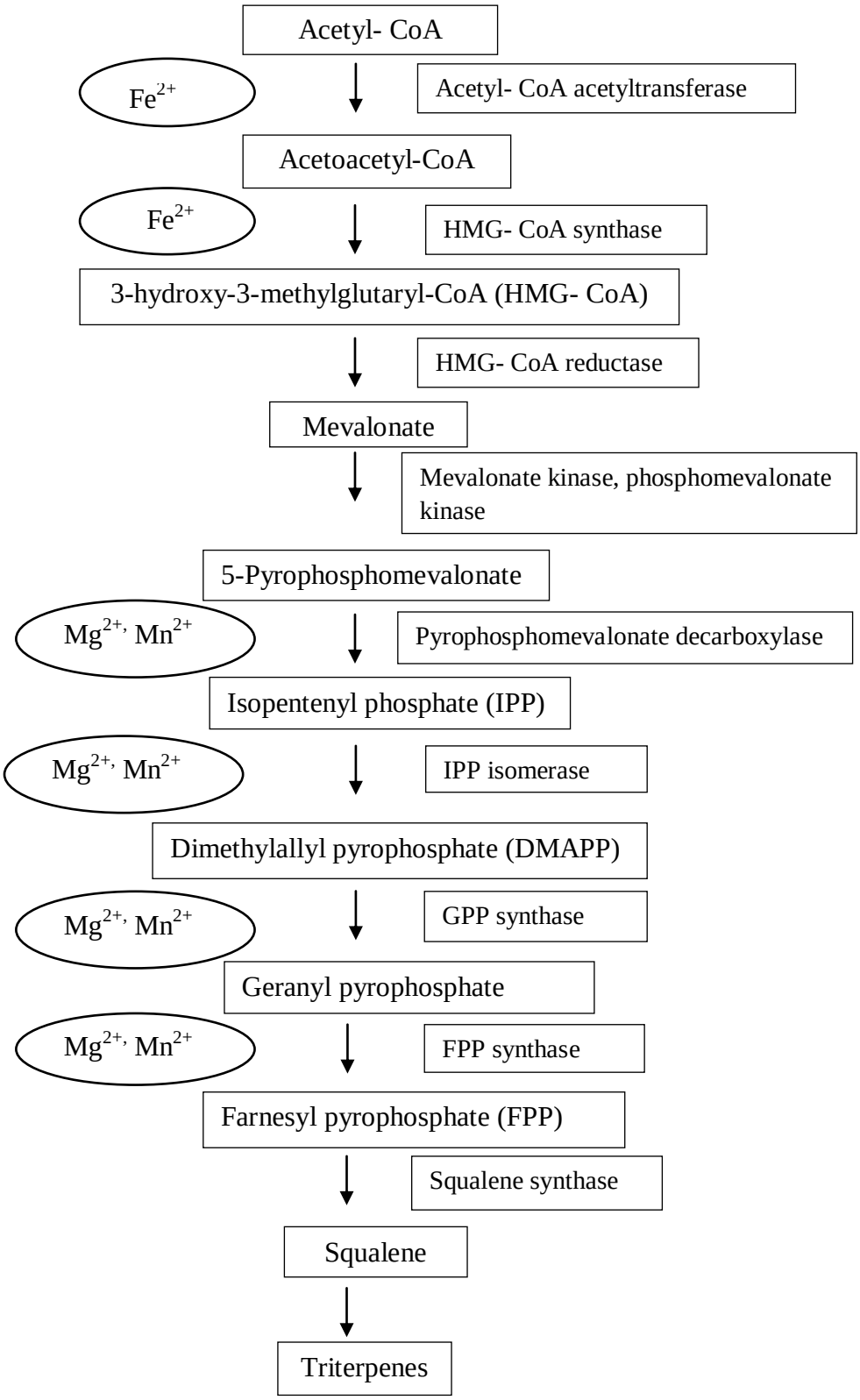

Fig. 4.1 Major steps in mevalonate pathway of triterpene biosynthesis modified from McGarvey and Croteau (1995)

However, the effects of pre-harvest spray application of $FeSO_4$ (Fe^{2+}), $MgSO_4$ (Mg^{2+}) or $MnSO_4$ (Mn^{2+}) on the levels of terpenoids and other phenolic compounds in ripe mango fruit were yet to be investigated. Based upon reported observations (Fischbach et al., 2000; Lucker et al., 2002; Rohdich et al., 2000) and the known fact that $Fe^{2+,}$ Mg^{2+} and Mn^{2+} act as cofactors of various enzymes involved in the biosynthesis of tepenoids (McGarvey and Croteau, 1995), it was hypothesized that pre-harvest spray application of $FeSO_4$ (Fe^{2+}), $MgSO_4$ (Mg^{2+}) or $MnSO_4$ (Mn^{2+}) may increase the levels of lupeol and carotenoids in ripe mango fruit due to up-regulation of terpenoid biosynthesis including chlorophyll. Further the increased levels of chlorophyll would possibly increase photosynthesis leading to higher production of phenolic compounds as polyphenols are biosynthesized via shikimic acid pathway that requires erythrose 4-phosphate and phosphoenol pyruvate generated during photosynthesis (Seigler, 1998). Therefore, the present study aimed at investigating the effects of pre-harvest spray application of different concentrations of $FeSO_4$, $MgSO_4$ and $MnSO_4$ on regulating the concentrations of terpenoids (lupeol and total carotenoids), mangiferin, phenolic acids, total phenols, total antioxidants and ascorbic acid in ripe mango fruit for the first time.

4.2 Materials and methods

Detailed analytical procedures and conditions are given in Chapter 3 (page 39).

4.2.1 Materials

Chemicals

$FeSO_4$ was purchased from Ajax Finechem (Auburn, NSW, Australia) while $MgSO_4$ was purchased from VWR International Pty Ltd. (Poole, England) respectively. $MnSO_4$ and the reagents and standards of lupeol, β-carotene, mangiferin and phenolic acids were purchased from Sigma Aldrich (St. Louis, MO, USA) and methanol, acetonitrile and n-hexane were purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Taren Point, NSW, Australia). HPLC grade reagents were used in the study.

Plant material and experimental treatments

Twenty seven years old 'Kensington Pride' mango trees of uniform size grown in a commercial orchard at Gingin (31° 27´S, 115° 55´E), Western Australia were used in this experiment. The spacing between trees was 5 m and the spacing between rows was 6.5 m. The row direction was East-West. All the trees used in this experiment received similar cultural practices including irrigation, plant protection and nutrition except the experimental treatments (Department of Agriculture and Food Western Australia, 2017). Aqueous solutions containing different concentrations (0.2% and 0.3%) of $FeSO_4$, $MgSO_4$ or $MnSO_4$ and Tween 20® as a surfactant (50.0 μL L^{-1}) were sprayed 30 d before anticipated harvest date (20th of February 2017). It was presumed that additional spray application of Fe, Mg and Mn 30 d before harvest will provide sufficient time to the fruit and leaves to uptake and metabolise these nutrients leading to upregulation of biosynthesis of terpenoids and phenolic compounds ensuring their higher levels in the fruit at harvest. Untreated trees served as control. The aqueous solution was sprayed onto the whole tree including leaves and fruit till run off. The concentrations of $FeSO_4$ (Kadman and Gazit, 1984), $MgSO_4$ (Xiuchong et al., 2001) and $MnSO_4$ (Papadakis et al., 2005) were chosen based upon previous reports. The experiment was laid out by following a randomized block design (RBD). A single tree was considered as an experimental unit and replicated 3 times. One month after the spray application of these nutrients, from each replication ten hard green mature fruit (light cream pulp, 100% green peel, firmness: 165±1 N) free from any defects were randomly harvested around the tree canopy and transported within 2 h to the Horticulture Research Laboratory, Perth, Western Australia. The fruit were allowed to ripen at ambient temperature (21±1.5 °C until eating soft stage (75% yellow peel). At eating soft stage, representative pulp and peel samples were prepared as described in Chapter 3, Section 3.2 and immediately stored at -80 °C for subsequent analysis of terpenoids (lupeol and total carotenoids), mangiferin, phenolic acids, total phenols, total antioxidant capacity and ascorbic acid. The concentrations of lupeol, mangiferin, and phenolic acids were determined using freeze-dried samples. The concentrations of total phenols, total antioxidants, total carotenoids and ascorbic acid were determined using thawed samples. Ten mangoes were included in each replication and replicated three times.

4.2.2 Determination of the concentrations of terpenoids

4.2.2.1. Lupeol

The methods previously described by Ruiz-Montañez et al. (2014) and Oliveira et al. (2012) with slight modifications as detailed in Chapter 3, Section 3.5.1 were followed for the extraction, identification and quantification of lupeol using an Agilent HPLC system with diode array detection at 210 nm. The concentration of lupeol in mango pulp/peel was calculated using a standard curve of lupeol and expressed as mg kg^{-1} dry weight basis.

4.2.2.2 Total carotenoids

The solvent extraction method described in Chapter 3, Section 3.5.6 was used to determine the total carotenoid concentration in the pulp of ripe mango using a UV/VIS spectrophotometer. The concentration of total carotenoids was calculated using a β-carotene standard curve and expressed as mg kg^{-1} fresh weight basis.

4.2.3 Determination of the concentrations of phenolic compounds

4.2.3.1 Total phenols

The method reported by Robles-Sànchez et al. (2009) was used to determine the total phenol concentration in the pulp and peel of ripe mango fruit using Folin-Ciocalteu reagent as detailed in Chapter 3, Section 3.5.3. The total phenol concentration was calculated using a gallic acid standard curve and expressed in g gallic acid equivalent (GAE) kg^{-1} fresh weight basis.

4.2.3.2 Mangiferin and phenolic acids

The concentrations of phenolic acids and mangiferin in the pulp and peel of mango fruit were determined using the method previously described by Palafox-Carlos et al. (2012a) using Agilent HPLC system with some modifications as detailed in Chapter 3, Section 3.5.2. The concentrations of polyphenols were quantified using standard curves of each compound and expressed as mg kg^{-1} dry weight basis.

4.2.4 Ascorbic acid

The concentration of ascorbic acid in the fruit pulp was determined at 760 nm using a UV/VIS spectrophotometer as described in Chapter 3, Section 3.5.5 and expressed as mg kg^{-1} fresh weight basis.

4.2.5 Total antioxidants

Total antioxidant capacity in the pulp and peel of ripe mango fruit was determined using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay as detailed in Chapter 3, Section, 3.5.4. The results were expressed as mmol Trolox equivalent antioxidant capacity (TEAC) kg^{-1} fresh weight basis.

4.2.6 Statistical analysis

Data were subjected to one-way ANOVA. Least significant differences were calculated at 5% level ($P \leq 0.05$). The treatment means were presented along with ± standard deviation (SD). Duncan multiple comparison test was used to used to calculate the significant differences among treatments. Genstat version 14.0 (Lawes Agricultural Trust, Rothamsted, UK) software was used to analyze the data statistically.

4.3 Results

4.3.1 Influence of the pre-harvest spray application of $FeSO_4$ (Fe^{2+}), $MgSO_4$ (Mg^{2+}) and $MnSO_4$ (Mn^{2+}) on the concentrations of terpenoids

4.3.1.1 Lupeol

Any of the pre-harvest spray treatments of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.2 % and 0.3 %) did not significantly affect the concentration of lupeol in the pulp of ripe fruit. However, it was highest in the pulp with the spray application of 0.3% $FeSO_4$ (9.85 mg kg^{-1}) (Fig. 4.2A). All the pre-harvest spray applications of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.2 % and 0.3 %) significantly increased the concentration of lupeol in the peel of ripe fruit compared to untreated control, whilst 0.2% $MgSO_4$ and 0.3% $MnSO_4$ treatments resulted in the highest concentrations (26.9 mg kg^{-1}, 27.1 mg kg^{-1} respectively) (Fig. 4.2B).

4.3.1.2 Total carotenoids

The concentration of total carotenoids in the pulp of ripe mango fruit was significantly increased ($P \leq 0.05$) with the pre-harvest application of 0.3% $FeSO_4$, $MgSO_4$ and $MnSO_4$ (64.4, 68.2 and 67.0 mg kg^{-1} respectively) compared to the untreated control (52.2 mg kg^{-1}) and all other pre-harvest spray treatments (Fig. 4.3). In general, higher concentration (0.3%) of $FeSO_4$, $MgSO_4$ or $MnSO_4$ applied as a pre-harvest spray was more effective in elevating the levels of total carotenoids in the pulp of ripe mango fruit as compared to the lower concentration (0.2%) applied.

4.3.2 Influence of the pre-harvest spray application of $FeSO_4$ (Fe^{2+}), $MgSO_4$ (Mg^{2+}) and $MnSO_4$ (Mn^{2+}) on the concentrations of phenolic compounds

4.3.2.1 Mangiferin and phenolic acids

The concentration of mangiferin in the pulp was higher in the ripe fruit when treated with 0.2% $FeSO_4$, $MgSO_4$ or $MnSO_4$ prior to harvesting compared to the untreated control and all other treatments (Fig. 4.4A). The pre-harvest spray application of these nutrients did not significantly affect the level of mangiferin in the peel of ripe fruit (Fig. 4.4B).

The concentrations of gallic and ferulic acids in the peel and chlorogenic acid in pulp of ripe fruit were significantly ($P \leq 0.05$) highest in the fruit sprayed with 0.2% $FeSO_4$ prior to harvesting compared to the untreated control and all the other treatments (Table 4.1). However, the pre-harvest spray application of nutrients did not significantly affect the concentrations of gallic and ferulic acids in the pulp and vanillic and caffeic acids in both the pulp and peel of ripe fruit compared to the untreated control (Table 4.1).

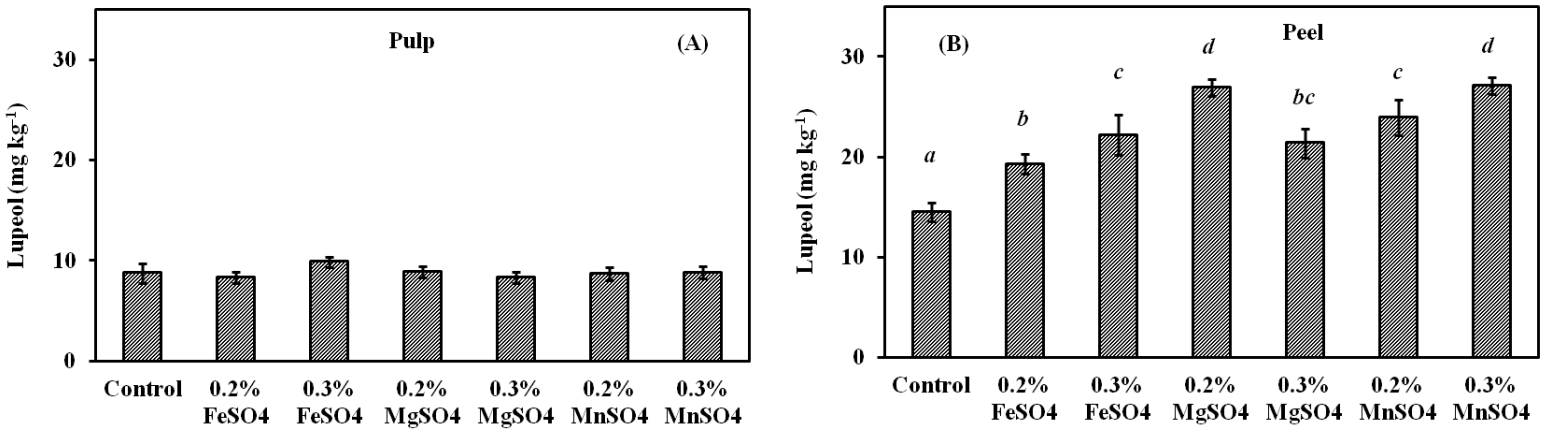

Fig. 4.2. Effects of pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ on the concentrations of lupeol in the pulp (A) and peel (B) of ripe mango fruit. Vertical bars represent standard deviation (SD) of means. Mean values significantly different at $P \leq 0.05$ level are indicated by different letters. Means followed by the same letter are not significantly different by Duncan multiple comparison test at $P \leq 0.05$ level.

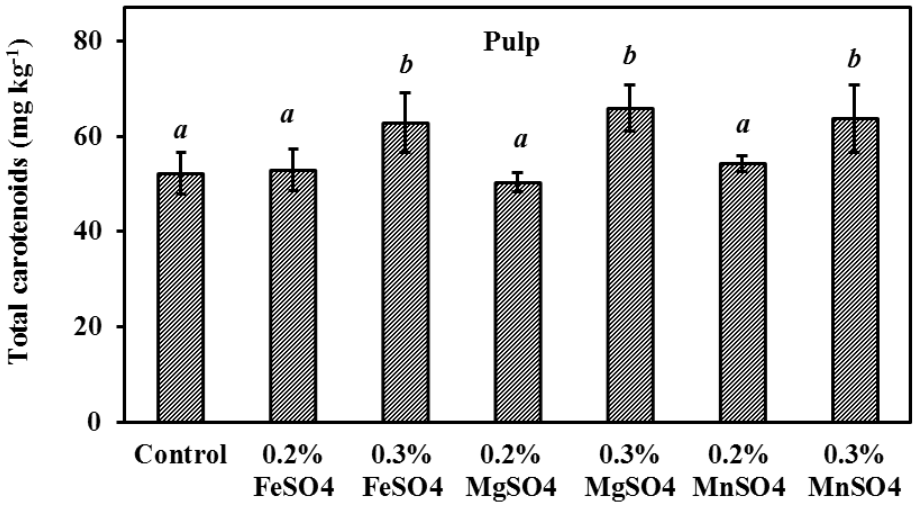

Fig. 4.3. Effects of pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ on the concentration of total carotenoids in the pulp of ripe mango fruit. Vertical bars represent standard deviation (SD) of means. Mean values significantly different at $P \leq 0.05$ level are indicated by different letters. Means followed by the same letter are not significantly different by Duncan multiple comparison test at $P \leq 0.05$ level.

4.3.2.2 Total phenols, ascorbic acid and total antioxidant capacity

Any of the pre-harvest spray applications of $FeSO_4$, $MgSO_4$ or $MnSO_4$ did not significantly affect the concentrations of total phenols and total antioxidant capacity in the pulp and peel of ripe mango fruit compared to the control (Table 4.2). Similarly, there was no significant effect of the pre-harvest spray applications of $FeSO_4$, $MgSO_4$ or $MnSO_4$ on the concentration of ascorbic acid in the pulp of ripe fruit (Table 4.2).

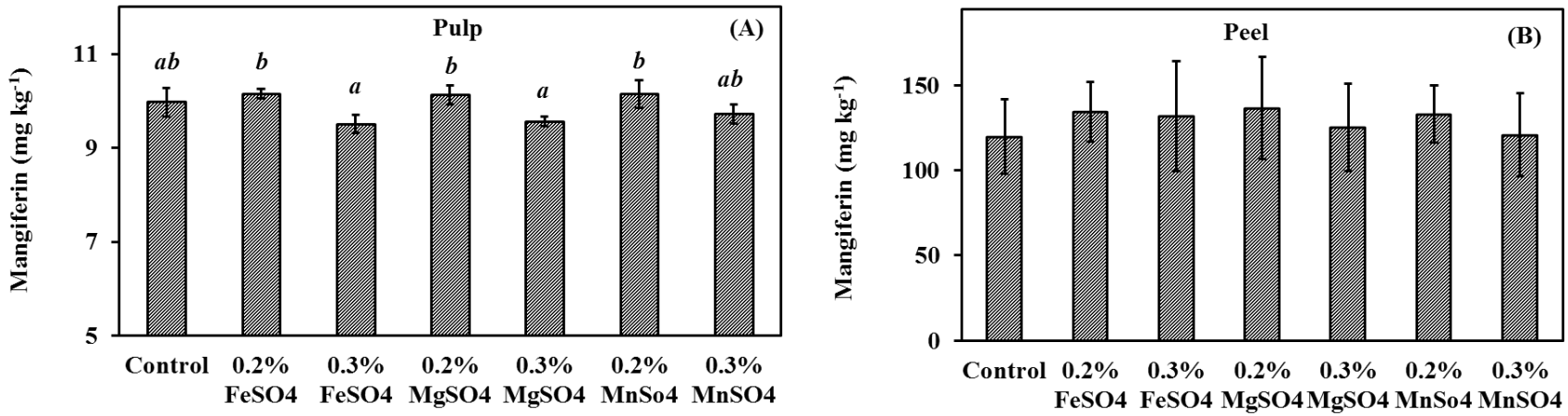

Fig. 4.4 Effect of pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ on the concentration of mangiferin in the pulp (A) and peel (B) of ripe mango fruit. Vertical bars represent standard deviation (SD) of means. Mean values significantly different at $P \leq 0.05$ level are indicated by different letters. Means followed by the same letter are not significantly different by Duncan multiple comparison test at $P \leq 0.05$ level.

Table 4.1. Effects of different concentrations of pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ on the concentrations of phenolic acids in the pulp and peel of ripe mango fruit

Treatment	Gallic acid (mg kg^{-1} DW)		Chlorogenic acid (mg kg^{-1} DW)		Vanillic acid (mg kg^{-1} DW)		Ferulic acid (mg kg^{-1} DW)		Caffeic acid (mg kg^{-1} DW)	
	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Control	173.0±24.1	4568.0±207.0^{a}	170.1±23.4^{a}	330.0±29.2	78.6±3.9	292.8±54.3	4.0±0.4	32.3±2.0^{ab}	1.9±0.5	14.4±3.3
$FeSO_4$ (0.2%)	203.1±42.6	5540.0±242.4^{b}	275.7±56.4^{b}	364.0±33.3	81.0±4.5	374.3±51.3	4.6±1.0	44.0±5.5^{c}	2.3±0.6	20.1±3.0
$FeSO_4$ (0.3%)	197.2±11.3	4163.0±451.5^{a}	145.3±11.3^{a}	324.4±20.6	77.0±6.9	297.0±44.1	3.7±0.3	25.5±4.4^{a}	2.2±0.5	16.5±3.7
$MgSO_4$ (0.2%)	209.3±79.5	4489.0±145.7^{a}	171.7±22.3^{a}	349.7±44.3	87.4±4.1	315.6±43.2	4.3±0.6	30.3±5.6^{b}	1.6±0.4	19.2±3.6
$MgSO_4$ (0.3%)	179.2±71.2	4307.0±267.7^{a}	153.0±13.4^{a}	264.7±53.0	78.6±6.4	276.5±46.1	3.7±0.8	32.6±3.2^{ab}	1.7±0.5	13.3±2.1
$MnSO_4$ (0.2%)	192.7±14.9	4291.0±285.8^{a}	178.1±16.0^{a}	343.5±36.8	89.6±6.9	323.2±14.3	4.5±0.7	32.9±2.9^{ab}	2.0±0.4	17.6±1.5
$MnSO_4$ (0.3%)	140.4±36.1	4133.0±259.9^{a}	152.3±10.3^{a}	298.4±28.5	84.1±10.0	321.1±40.7	4.0±0.4	34.7±5.1^{b}	1.4±0.4	14.9±2.9

DW = Dry weight, n = 3 ± SD, 10 fruit per replication, Mean values significantly different at $P \leq 0.05$ are indicated by different lower case, superscript letters along each column. No lettering along a column denotes non significance at $P \leq 0.05$.

Table 4.2. Effects of different concentrations of pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ on the concentrations of total phenols, ascorbic acid, and total antioxidant capacity in pulp and/or peel of ripe mango fruit

Treatment	Total phenols (g GAE kg^{-1} FW)		Ascorbic acid (mg kg^{-1} FW)	Total antioxidant capacity (mmol TEAC kg^{-1} FW)	
	Pulp	Peel	Pulp	Pulp	Peel
Control	6.4± 0.2	95.8±13.4	498.2±28.0	1.7±0.05	52.0±1.8
$FeSO_4$ (0.2%)	7.5±0.8	111.6±9.7	525.0±21.9	1.7±0.06	51.7±1.2
$FeSO_4$ (0.3%)	3.1±0.7	135.5±19.7	502.1±23.2	2.0±0.17	51.1±3.9
$MgSO_4$ (0.2%)	9.2±0.2	105.9±7.0	481.2±38.9	1.8±0.12	51.9±0.7
$MgSO_4$ (0.3%)	7.3±0.2	99.5±23.5	402.9±11.7	1.9±0.02	56.1±1.2
$MnSO_4$ (0.2%)	6.1±0.8	119.4±18.6	509.8±14.6	1.8±0.2	53.8±3.1
$MnSO_4$ (0.3%)	7.4±0.2	120.4±21.9	505.7±47.6	1.9±0.16	55.5±5.3

FW = Fresh weight, n = 3 ± SD, 10 fruit per replication, No lettering along a column denotes non significance at $P \leq 0.05$.

4.4 Discussion

The concentrations of lupeol in the peel of ripe mango fruit was significantly ($P \leq 0.05$) increased with the pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.2% and 0.3%) compared to the control. The concentration of total carotenoids in the pulp of ripe fruit was significantly increased with the pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.3%) compared to control and all other treatments. The increase in the concentrations of lupeol and total carotenoids might be a result of the increased activity of enzymes involved in the terpenoid biosynthesis process. The production of precursors of triterpenes (e.g. lupeol) and tetraterpenes (e.g. carotenoids) is stimulated by acetyl CoA acetyltransferase and HMG- CoA synthase by the supply of bivalent metal ions Fe^{2+} and pyrophosphomevalonate decarboxylase, IPP isomerise, GPP synthase and FPP synthase by the supply of bivalent metal ions Mg^{2+} and Mn^{2+} through pre- harvest mineral nutrients application (McGarvey and Croteau, 1995) (Fig. 4.1). It may also be argued that the increased level of photosynthesis due to increased production of chlorophylls via induced terpenoid biosynthesis (McGarvey and Croteau, 1995). The activation of the process of photosynthesis results in higher production of carbohydrates which generates the precursors of acetyl- CoA: phosphoenol pyruvate and pyruvate (Seigler, 1998). As the conversion of acetyl- CoA to acetoacetyl- CoA is the first step of terpenoid biosynthesis (Fig.4.1), this also might have contributed to the increased production of lupeol and total carotenoids. Earlier, Fischbach et al. (2000) reported that the activity of monoterpene synthase was induced with different preferences for Mg^{2+} and Mn^{2+} as cofactors in the biosynthesis of monoterpenes in Norway spruce. In agreement, the pre-harvest spray application of $MgSO_4$ and $MnSO_4$ (0.2% and 0.3% respectively) increased the concentration of lupeol (triterpene) in the peel of ripe mango fruit. However, the activity of monoterpene synthase in holm oak leaves was strongly dependent on Mg^{2+} and the replacement of Mg^{2+} with Mn^{2+} significantly reduced the activity of this enzyme. Moreover, the addition of Mn^{2+} as bivalent metal ion cofactor increased the activity of monoterpene synthase enzyme in lemon (Lucker et al., 2002), whereas the terpenoid biosynthesis was induced by the addition of Mg^{2+} in tomato (Rohdich et al., 2000). These observations indicate that the

preferences over bivalent metal ions could be genotype and pulp and peel tissue dependent.

The concentrations of gallic and ferulic, acids in the peel and chlorogenic acid in pulp of the ripe mango fruit were significantly increased with the spray application of 0.2% $FeSO_4$ compared to the untreated control and other treatments (Table 4.1). The concentration of mangiferin in the pulp of ripe fruit was significantly highest with the pre-harvest application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (0.2%) compared to control and all other treatments (Fig. 4.4A). The increase in these phenolic compounds might be a result of the increased levels of photosynthesis due to higher production of chlorophyll as a result of terpenoid biosynthesis activation as mentioned earlier. Because, phosphoenol pyruvate and erythrose 4- phosphate generated during photosynthesis are precursors to the shikimic acid biosynthetic pathway of phenolic acids (Seigler, 1998). The increased soluble solids concentration (%) in the juice of ripe mango fruit with the pre-harvest application of 0.2% and 0.3% $FeSO_4$ (16.8% and 17.1% respectively), 0.2% and 0.3% $MgSO_4$ (18.4% and 17.6% respectively) and 0.2% and 0.3% $MnSO_4$ (18.1% and 16.6% respectively) compared to control fruit (15.7%) (data not shown) indirectly hints an enhanced rate of photosynthesis with the pre- harvest application of these mineral nutrients. However, the concentrations of vanillic and caffeic acids in both pulp and peel, gallic and ferulic acids in the pulp, chlorogenic acid and mangiferin in the peel of ripe mango fruit were not significantly influenced with the pre-harvest application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ (Fig. 4.4B, Table 4.1 and Table 4.2). These observations suggest that, the influence of the pre- harvest spray application of mineral nutrients on the concentration of phenolic compounds in mango fruit could be compound and tissue dependent.

4.5 Conclusion

All the pre-harvest spray applications ($FeSO_4$, $MgSO_4$ and $MnSO_4$) significantly increased the concentration of lupeol in the peel of ripe fruit compared to untreated control, whilst the 0.2% $MgSO_4$ and 0.3% $MnSO_4$ treatments were most effective. Pre-harvest spray application of $FeSO_4$, $MgSO_4$ or $MnSO_4$ (0.3%) increased the

concentration of total carotenoids in the pulp of ripe fruit. In general, 0.2% $FeSO_4$ pre-harvest spray treatment could be considered as a simple and practical method to increase the levels of phenolic health-promoting compounds in ripe mango fruit. Monitoring the effects of pre-harvest spray application of $FeSO_4$, $MgSO_4$ or $MnSO_4$ on the activity of the enzymes involved in the biosynthetic pathway of terpenoids would provide a better insight into the findings in future investigations.

CHAPTER 5

Harvest maturity stage affects the concentrations of health-promoting compounds: lupeol, mangiferin and phenolic acids in the pulp and peel of ripe 'Kensington Pride' mango fruit

ABSTRACT

Mango (*Mangifera indica* L.) fruit is known as a good source of lupeol, mangiferin and phenolic acids. However, the effect of harvest maturity on the concentrations of these compounds in the pulp and peel of ripe 'Kensington Pride' mango fruit has not been reported. Thus, quantitative analysis of lupeol, mangiferin, phenolic acids and other important health-promoting compounds in the pulp and peel of ripe mango fruit harvested at four different maturity stages namely, green mature (commercial standard), sprung stage, half ripe and tree ripe was carried out. The highest concentrations of lupeol, mangiferin, vanillic acid, ferulic acid and caffeic acid in both pulp and peel and gallic acid, chlorogenic acid and total phenols in the peel were recorded in ripe fruit harvested at the sprung stage. The highest concentrations of ascorbic acid and total carotenoids in pulp and total antioxidant capacity in peel were recorded in the fruit harvested at tree ripe stage, whilst the highest antioxidant capacity in ripe pulp was recorded in those fruit harvested at half ripe stage. The sprung stage could, therefore, be considered as the best stage to harvest to obtain mango fruit with enhanced health benefits.

KEYWORDS: *Mangifera indica* L.; Harvest maturity; health-promoting compounds; lupeol; mangiferin; phenolic acids

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5.1 Introduction

Nutrition recommendations would provide a feasible mean to overcome the economic burden of current health issues worldwide (Kruger et al., 2014). Based on the findings of many clinical and epidemiological studies, polyphenols present in fruit and vegetables are potential compounds that can reduce the risk of several degenerative diseases (Scalbert et al., 2005). Mango (*Mangifera indica* L.) is a good dietary source of polyphenols, antioxidants, vitamins and carotenoids (Masibo and He, 2009). Mango fruit was also recognized as a good source of lupeol in the recent past (Gallo and Sarachine, 2009). Lupeol is a pentacyclic triterpene with a potential to act as an anti-inflammatory, anti-invasive, and cholesterol-lowering agent with a significant ability to reduce the risk of a range of chronic conditions (Siddique and Saleem, 2011) including its ability to target diseased human cells without causing any damage to the healthy cells (Saleem, 2009). Mangiferin is another important compound found in mango with a significant medicinal value (Masibo and He, 2008). Its ability to act as an anticancer compound is well documented (Masibo and He, 2009). Mango is also rich in phenolic acids such as gallic acid, chlorogenic acid, vanillic acid, ferulic acid and caffeic acid among several others. Gallic acid is identified as the major phenolic acid in mango fruit with a strong antioxidant potential (Kim et al., 2007; Yen et al., 2002). In several studies, it was identified as the principal constituent in plant extracts that inhibit the growth of human prostate carcinoma cells (Veluri et al., 2006; Chen et al., 2009). Based on several *in vivo* and *in vitro* studies, chlorogenic acid was also found to be capable of exhibiting important antioxidant and anti-carcinogenic activities (Farah et al., 2008). Similarly, ferulic acid is a common phenolic compound that is considered as one of the key ingredients in indigenous Chinese medicine (Ou and Kwok, 2004).

The amount of phenolic compounds in fruit is strongly dependent on the degree of ripeness (Belitz et al., 2004). Mango fruit is generally harvested at the green mature stage for maximum storage life and for easier handling and transportation due to its higher firmness (Lalel et al., 2003a). Presently, consumers are placing more emphasis on the concentration of health-promoting compounds in fruit and vegetables and may forfeit the external appearance (Schreiner and Huyskens-Keil,

2006). This new trend in demand opens opportunities to modify current harvesting practices to increase the concentration of these compounds to gain the maximum health benefits.

Palafox-Carlos et al. (2012a, 2012b) harvested mangoes at four different maturity stages (R1- 0-10%, R2- 11-40%, R3- 41-70% and R4- 71-100% yellow surface) and immediately estimated the concentrations of major phenolic compounds, total phenols and total antioxidant potential in the pulp of 'Ataulfo' mangoes without postharvest ripening. Similarly, the concentrations of lupeol and mangiferin were also previously estimated in 'Ataulfo' mangoes immediately after harvesting at approximately green mature (physiological maturity) and eating soft (consumption maturity) stages (Ruiz-Montanez et al., 2014). 'Kensington Pride' is the most widely grown and available cultivar of mangoes in Australia which accounts for about 55% of the total mango production (Horticulture Innovation Australia Limited, 2017). Lalel et al. (2003a) reported the effect of harvest maturity on physicochemical parameters such as colour, firmness, soluble solids concentration, acidity, carotenoid levels and aroma volatile compounds in 'Kensington Pride' mangoes after postharvest ripening. However, there have been no reports on the influence of harvest maturity on the concentrations of lupeol, mangiferin and polyphenol profile in the pulp and peel of ripe fruit of any Australian mango cultivar. It was hypothesized that fruit maturity stage at harvest will influence the concentrations of health-promoting compounds in the pulp and peel of ripe mango fruit. Therefore, the influence of different harvest maturity stages on the concentrations of health-promoting compounds such as lupeol, mangiferin, phenolic acids, total phenols and total antioxidants in the pulp and peel as well as carotenoids, ascorbic acid in the pulp of ripe 'Kensington Pride' mango fruit was investigated.

5.2 Materials and methods

The detailed methods and condition are given in Chapter 3 (page 39).

5.2.1 Materials

Fruit

'Kensington Pride' mango fruit were harvested at four different maturity stages from a commercial orchard in Gingin (31° 27´S, 115° 55´E), Western Australia and transported within 2-3 h to the Curtin Horticulture Research Laboratory, Curtin University, Perth, Western Australia on the 9th of March 2015. Subjective evaluation of peel and pulp colour and the firmness were used to determine the stage of maturity as described earlier (Lalel et al., 2003a); namely, green mature (light cream pulp, green peel, hard), sprung stage (cream pulp, green peel, springy), half ripe (yellow pulp, 50-60% yellow peel, slightly soft) and tree ripe (yellowish orange pulp, >75% yellow peel, eating soft). Mango fruit of uniform size, free from any visual symptoms of disease (s) and physical injuries were used in this experiment. Fruit belonging to other maturity stages (except tree-ripe fruit) were allowed to ripen until eating soft stage (yellowish orange pulp and >75% yellow peel) at ambient temperature (21±1.5 °C). Tree-ripe fruit immediately and fruits form other treatments at eating soft stage were separated into representative pulp and peel samples and stored as described in Chapter 3, Section 3.2. Freeze-dried and powdered samples were used for the determination of the concentrations of lupeol, mangiferin, and phenolic acids. Thawed fresh samples were used to quantify the total phenols, total antioxidants, total carotenoids and ascorbic acid. The experiment was laid out as one factor factorial completely randomized design with four replications. Ten mangoes were included in each replication.

Chemicals

Acetonitrile, n-hexane, and methanol were purchased from Thermo Fisher Scientific (Taren Point, NSW, Australia). All the standards and the other reagents were

purchased from Sigma Aldrich (St. Louis, MO, USA). All the purchases were of HPLC grade.

5.2.2 Determination of health-promoting compounds in ripe mango fruit

5.2.2.1 Lupeol

Freeze-dried and powdered pulp/peels of representative mango samples were used to extract and quantify lupeol using the methods reported by Ruiz-Montañez et al. (2014) and Oliveira et al. (2012) with some modifications as detailed in Chapter 3, Section 3.5.1. The concentration of lupeol was expressed as mg kg^{-1} dry weight basis using a standard curve.

5.2.2.2 Mangiferin and phenolic acids

The polyphenol profile in freeze-dried and powdered pulp and peel samples of ripe mango fruit was determined using the method reported by Palafox-Carlos et al. (2012a) with some modifications stated in Chapter 3, Section 3.5.2. The levels of mangiferin and individual phenolic acids (gallic, chlorogenic, vanillic, ferulic and caffeic) were calculated using the corresponding standard curves and expressed as mg kg^{-1} dry weight basis.

5.2.2.3 Total phenols

The concentration of total phenols was determined by the method reported by Robles-Sànchez et al. (2009) using Folin-Ciocalteu reagent with some alterations detailed in Chapter 3, Section, 3.5.3. The quantification of total phenol concentration was done using a gallic acid standard curve and the concentrations of total phenols in the pulp and peel were expressed in g gallic acid equivalents (GAE) kg^{-1} fresh weight basis.

5.2.2.4. Total antioxidants

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was followed to determine the total antioxidant capacity of the representative samples of ripe mango pulp and peel as described in Chapter 3, Section, 3.5.4. The total antioxidant capacity calculated based on a standard curve of Trolox (6-hydroxy-2, 5, 7, 8-tetramethychromane-2-carboxylic acid) was expressed as mmol Trolox equivalent antioxidant capacity (TEAC) kg^{-1} fresh weight basis.

5.2.2.5 Ascorbic acid

The ascorbic acid concentration was estimated using a UV/VIS spectrophotometer (Jenway spectrophotometer, Model 6405) following the method reported by Malik and Singh (2005) as described in Chapter 3, Section, 3.5.5. The ascorbic acid concentration computed using a standard curve of L-ascorbic acid was expressed as mg kg^{-1} fresh weight basis.

5.2.2.6 Total carotenoids

The total carotenoids were extracted from the samples of ripe fruit pulp using the method reported by Lalel et al. (2003a) as detailed in Chapter 3, Section 3.5.6 under reduced light conditions. The total carotenoid concentration calculated using a β-carotene standard curve was expressed as mg kg^{-1} fresh weight basis.

5.2.3 Statistical analysis

The statistical analysis of the experimental data was carried out using one-way ANOVA with GenStat version 14.0 (Lawes Agricultural Trust, Rothamsted, UK) software. To compare significant differences among various treatments, the Duncan multiple comparison test was used. The values were expressed as means ± standard deviation of the mean (SD).

5.3 Results

5.3.1 The effect of maturity at harvest on the concentrations of health-promoting compounds

5.3.1.1 Lupeol

The concentrations of lupeol in the pulp and peel of ripe mango fruit were significantly affected by the maturity level at harvest ($P \leq 0.05$). A significantly lower concentration of lupeol was observed in the pulp from the fruit harvested at green mature stage (9.31 mg kg^{-1}) compared to the other three stages, However, the highest concentration of lupeol in the peel (24.6 mg kg^{-1}) was recorded in ripe fruit harvested at sprung stage (Fig. 5.1A and 5.1B). Significantly lower concentrations of lupeol were noted in the ripe peel of mangoes harvested at green mature (16.9 mg kg^{-1}) and tree ripe stages (16.42 mg kg^{-1}) (Fig. 5.1B). Overall, the concentration of lupeol in the ripe peel was approximately two-fold higher than pulp.

5.3.1.2 Mangiferin

The concentrations of mangiferin in the pulp and peel were significantly affected by the maturity at harvest ($P \leq 0.05$) (Fig. 5.1C and 5.1D). The highest concentration of mangiferin in the ripe pulp (13.2 mg kg^{-1}) was found in the fruit harvested at sprung stage (Fig. 5.1C). Significantly higher concentrations of mangiferin were recorded in the peel of fruit harvested at sprung (196.4 mg kg^{-1}) and green mature stages (178.7 mg kg^{-1}) compared to the other stages (Fig. 5.1D). The concentration of mangiferin in the peel was approximately 12to15 times higher than the pulp regardless of the harvest maturity.

5.3.1.3 Total phenols

The concentrations of total phenols in the pulp and peel of the ripe mangoes were significantly affected by the maturity at harvest ($P \leq 0.05$). The highest concentration of total phenols in the ripe pulp (9.3 g GAE kg^{-1}) was found in the mango fruit harvested at sprung stage (Fig. 5.2A). However, the highest concentration of total phenols in the peel (168.0 g GAE kg^{-1}) was found in the fruit harvested at tree ripe stage and the lowest was from the fruit harvested at green mature stage (88.1 g GAE kg^{-1}) (Fig. 5.2B). The concentration of total phenols in the peel was between 14-35 times more than pulp.

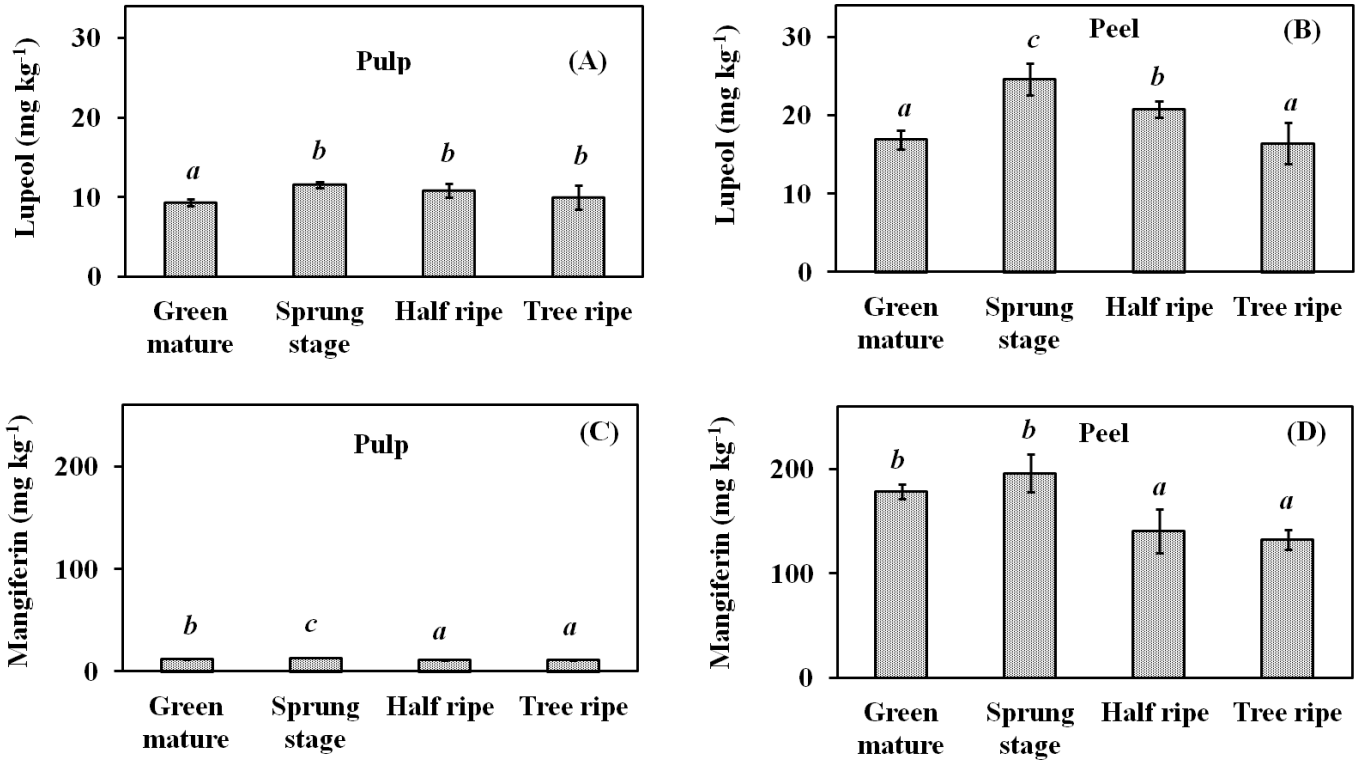

Fig. 5.1 The concentrations of lupeol in the pulp (A) and peel (B) and mangiferin in the pulp (C) and peel (D) of ripe mango fruit harvested at different maturity stages. n = 4, 10 fruit per replication. Vertical bars represent standard deviation (SD) of means. Mean values significantly different at $P \leq 0.05$ are indicated by different letters.

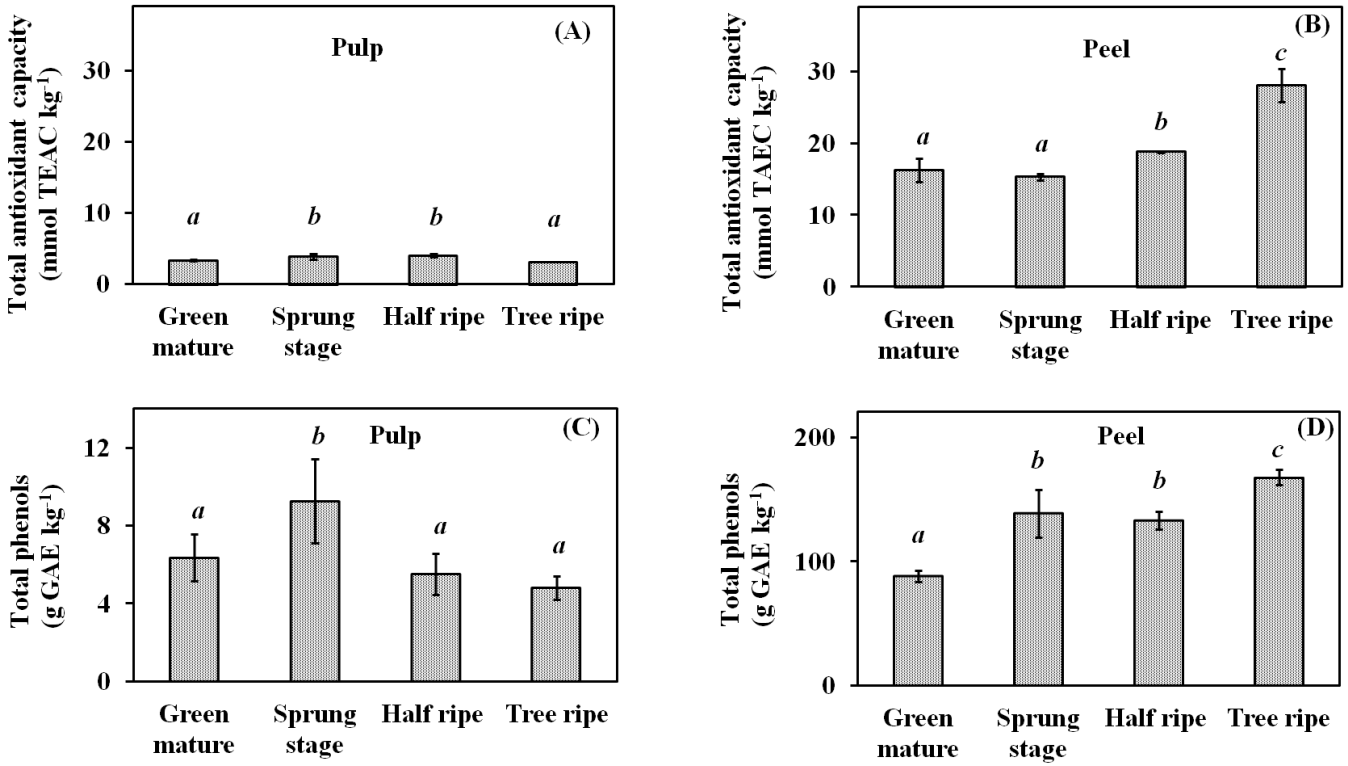

Fig. 5.2 The concentrations of total phenols in the pulp (A) and peel (B) and the total antioxidant capacity in the pulp (C) and peel (D) of ripe mango fruit harvested at different maturity stages. n = 4, 10 fruit per replication. Vertical bars represent standard deviation (SD) of means. Mean values significantly different at $P \leq 0.05$ are indicated by different letters.

5.3.1.4 Phenolic acids

Gallic, chlorogenic and vanillic acids were identified as the major phenolic acids in both the pulp and peel of ripe mango fruit (Table 5.1). The concentrations of all these compounds in both the pulp and peel were significantly affected by the maturity at harvest except the concentration of chlorogenic acid in the ripe pulp ($P \leq 0.05$). The highest concentration of gallic acid was found in the pulp of the fruit harvested at the green mature stage while the highest concentration of vanillic acid was recorded in the ripe pulp of fruit harvested at the sprung stage. The concentrations of gallic acid, chlorogenic acid, and vanillic acid were significantly highest in the peel of the fruit harvested at sprung stage compared to all other stages. The concentration of chlorogenic acid was about 3.0 to 3.5fold higher in the ripe peel than pulp, while that of vanillic acid was 4.5 to 6.0 fold higher in the ripe peel. The concentration of gallic acid was far higher (30 to 50 times) in the ripe peel than pulp. Ferulic acid and caffeic acid were the minor phenolic acids identified in the ripe pulp and peel (Table 5.1). The concentrations of these compounds in ripe pulp and peel were significantly affected by the maturity at harvest ($P \leq 0.05$), except the concentration of caffeic acid in the ripe peel. The concentration of ferulic acid in the pulp of fruit harvested at green mature and sprung stages were significantly higher than half ripe and tree ripe stages. The concentration of ferulic acid was the lowest in the ripe peel of the fruit harvested at tree ripe stage and the highest in those harvested at the sprung stage.

5.3.1.5 Total antioxidant capacity

The total antioxidant capacity of both pulp and peel of the ripe mango fruit was significantly affected by the maturity at harvest ($P \leq 0.05$). The highest total antioxidant capacity was noted in the ripe pulp of the fruit harvested at the half-ripe (4.0 mmol Trolox kg^{-1}) and the sprung stages (3.9 mmol Trolox kg^{-1}) (Fig. 5.2C). In contrast, the highest total antioxidant capacity in the peel was found when harvested at tree ripe stage (28.0 mmol Trolox kg^{-1}) and the lowest in the fruit harvested at green mature stage (16.2 mmol Trolox $kg^{-1)}$ and sprung stage (15.3 mmol Trolox kg^{-}

^{1}) (Fig. 5.2D). The total antioxidant capacity of the peel was 4-9 times higher than the pulp.

5.3.1.6 Ascorbic acid and total carotenoids

The concentration of ascorbic acid in the ripe pulp of the mango fruit was not significantly affected by the maturity stage at harvest ($P \leq 0.05$) (Fig. 5.3A). However, the influence of maturity stage at harvest was significant on the concentration of total carotenoids in the ripe pulp ($P \leq 0.05$). A significantly higher concentration of total carotenoids (46.6 mg kg^{-1}) was noted in the pulp of the fruit harvested at tree ripe stage when compared with the fruit harvested at the other stages of maturity (Fig. 5.3B).

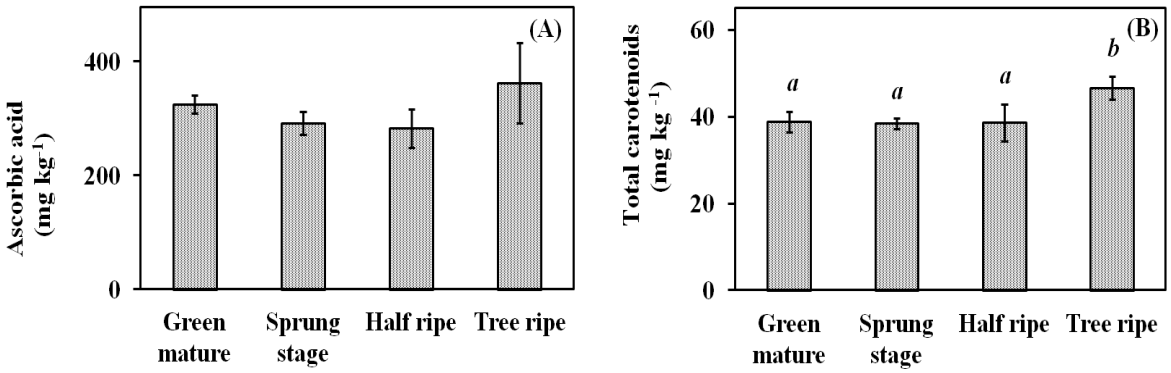

Fig. 5.3 The concentrations of ascorbic acid (A) and total carotenoids (B) in the pulp of ripe mango fruit harvested at different maturity stages. n = 4, 10 fruit per replication. Vertical bars represent standard deviation (SD) of means. Mean values significantly different at $P \leq 0.05$ are indicated by different letters. No lettering denotes non-significance.

Table 5.1. The concentrations of phenolic acids in the pulp and peel of ripe mango fruit harvested at different maturity stages

Harvest maturity	Gallic acid (mg kg $^{-1}$ DW)		Chlorogenic acid (mg kg $^{-1}$ DW)		Vanillic acid (mg kg $^{-1}$ DW)		Ferulic acid (mg kg $^{-1}$ DW)		Caffeic acid (mg kg $^{-1}$ DW)	
	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Green mature	151.3±32.6^{c}	6667.5±200.0^{c}	130.2±13.4	338.4±13.7^{a}	108.3±14.8^{b}	487.2±28.2^{c}	4.1±0.5^{b}	37.8±4.1^{b}	2.9±0.2	19.1±1.9
Sprung stage	118.9±6.2^{b}	7682.7±192.0^{d}	126.6±16.1	446.9±59.6^{b}	211.6±20.0^{c}	524.0±32.0^{c}	4.9±0.5^{b}	45.1±4.5^{b}	3.0±0.3	19.8±1.9
Half ripe	95.0±9.7^{b}	4644.8±626.5^{b}	113.3±10.3	298.7±33.6^{a}	75.3±6.9^{a}	458.8±36.5^{b}	2.3±0.4^{a}	38.1±3.3^{b}	1.8±0.3	19.1±2.0
Tree ripe	86.0±7.1^{a}	3863.2±493.1^{a}	106.6±9.1	295.8±42.6^{a}	74.8±8.5^{a}	393.2±33.3^{a}	2.2±0.4^{a}	32.5±3.6^{a}	1.9±0.3	18.4±2.2

DW = Dry weight, n = 4 ± SD, 10 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by different lower-case, superscript letters within a column. No lettering along a column denotes non-significance.

5.4 Discussion

The storage life and quality of ripe fruit are greatly influenced by the maturity stage at harvest (Kader, 1999). The concentration of phenolic compounds in fruit is also highly dependent on the degree of ripeness (Belitz et al. 2004). In this study, the concentrations of lupeol (Fig. 5.1A, 5.1B) and mangiferin (Fig. 5.1C, 5.1D) in ripe pulp and peel of 'Kensington Pride' mangoes were significantly affected by the stage of maturity at the time of harvest. Similarly, Ruiz-Montanez et al. (2014) also reported that significantly higher concentrations of these two compounds were noted in the pulp and peel of 'Ataulfo' mangoes harvested at eating soft stage (consumption maturity) when compared with the fruit harvested at green mature stage (physiological maturity) immediately after harvest. As previously pointed out by other authors, the concentrations of lupeol and mangiferin were considerably higher in the peel of the ripe fruit than the pulp (Srivastava et al., 2015; Ruiz-Montanez et al., 2014). The difference of the concentrations of lupeol and mangiferin in pulp and peel of 'Ataulfo' mango was in the range of 1 - 4 times (Ruiz-Montanez et al., 2014) whereas; it was in the range of 2 - 43 times (mangiferin) and 1-40 times (lupeol) in Indian mangoes (Srivastava et al., 2015). Polyphenols and terpenoids including lupeol and mangiferin are produced as secondary metabolites in plants, thus their levels are greatly influenced by genetic and environmental factors (Bourgaud et al., 2001). Therefore; the stage of maturity of the plant tissue and its exposure to biotic and abiotic stresses can directly influence the genes that express the metabolic enzymes which are required for the synthesis of these compounds (Bourgaud et al., 2001).The higher concentration of these compounds in the peel could be due to their role in defense against various biotic and abiotic stress conditions during fruit development (Ribeiro et al., 2008).

The degree of exposure to environmental stresses (average 35 °C and 50-55% relative humidity) was likely the highest in those mango fruit harvested at tree ripe stage in the present study. Thus, a continuous stimulation might have occurred for the production of polyphenolic compounds leading to the highest concentration of total phenols in the peel of tree-ripe fruit (Fig. 5.2B).

In agreement with the current study, gallic acid was identified as the main phenolic compound found in the pulp of 'Tommy Atkins' (Kim et al., 2007), whilst chlorogenic acid was identified as the major phenolic acid in the pulp of 'Ataulfo' mangoes, followed by gallic acid and vanillic caid (Palafox-Carlos et al., 2012a). The production and retention of different phenolic acids in mango fruit at different stages of maturity are apparently cultivar specific (Kim et al., 2007). In the present study, the concentration of gallic acid was significantly influenced by the harvest maturity. This contrasts with 'Ataulfo' mango pulp. In agreement with our findings, the concentrations of chlorogenic acid and vanillic acid were significantly high in 'Ataulfo' mango fruit harvested at ripe stage (Palafox-Carlos et al., 2012a). This highlights that the changes in the polyphenol profile are cultivar dependent.

In mango fruit, the total antioxidant capacity is contributed mainly by phenols and ascorbic acid (Li et al., 2014; Ma et al., 2011; Martinez et al., 2012; Talcott et al., 2005). The significantly high total antioxidant capacity in the ripe pulp of mango fruit harvested at sprung and half ripe stages (Fig. 5.2C) can be attributed to the high concentrations of phenolic compounds. The significantly high concentration of total antioxidants reported in the peel of mangoes harvested at tree ripe stage (Fig. 5.2D) could be a combined result of higher concentrations of polyphenols and ascorbic acid. In agreement with previous reports (Dorta et al., 2012; Kim et al., 2010a) the peel showed a higher antioxidant capacity than pulp across all maturity stages (Fig. 5.2D).

5.5 Conclusion

The highest concentrations of health-promoting compounds: lupeol, mangiferin, vanillic acid, gallic acid and chlorogenic acid were recorded in the pulp and /or peel of ripe mango fruit which were harvested at the sprung stage. Thus, altering the current standard of maturity at harvest from green mature to sprung stage may create opportunities to produce ripe mango fruit with greater health benefits. The peel of ripe fruit harvested at the sprung stage could be used as a promising source for the extraction of these desirable compounds. Future studies on storage life of mango fruit harvested at later maturity stages and methods to maintain quality aspects of these

fruit until they reach consumers would be beneficial to reduce postharvest losses of this potentially more health-beneficial product.

CHAPTER 6

Dynamics in the concentrations of health-promoting compounds: lupeol, mangiferin and different phenolic acids during postharvest ripening of mango fruit

ABSTRACT

Mango fruit (*Mangifera indica* L.) is renowned for its pleasant taste and as a rich source of health beneficial compounds. The aim of this study was to investigate the changes in concentrations of health-promoting compounds *viz.*, ascorbic acid, carotenoids, antioxidants, lupeol, mangiferin, total phenols, individual phenolic acids as well as ethylene production and respiration rates during climacteric ripening in 'Kensington Pride' and 'R2E2' mango fruit. The climacteric ethylene and respiration peaks were noted on the third day of fruit ripening period. The concentrations of total carotenoids in the pulp, total antioxidants in both pulp and peel, total phenols of the peel, lupeol, and mangiferin were significantly elevated, whilst the concentration of ascorbic acid declined during post-climacteric ripening. Gallic, chlorogenic and vanillic acids were identified as the major phenolic acids in both pulp and peel of 'Kensington Pride' and 'R2E2' mangoes. The concentrations of phenolic acids (gallic, chlorogenic, vanillic, ferulic and caffeic acids) also increased during the post-climacteric phase. The concentrations of all phenolic compounds were several-fold higher in the peel than pulp. Mangoes at post-climacteric ripening phase offer the highest concentrations of health-promoting compounds. Peel, at this stage of fruit ripening could be exploited as a good source for extraction of these compounds.

KEYWORDS: *Mangifera indica* L.; fruit ripening; health-promoting compounds; lupeol; mangiferin; phenolic acids

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6.1 Introduction

There is growing evidence of the protective role of plant bioactive compounds in reducing the risk of degenerative diseases such as cancer which has given hope for better health prospects through an increased consumption of fruit and vegetables (Scalbert et al., 2005). For instance, one-third of all cancers and approximately half of the cardiovascular disease incidences are caused by behavioural and dietary risks according to the World Health Organization (WHO 2017a and 2017b).

Mango (*Mangifera indica* L.) is a fruit with a significantly high level of health-promoting compounds such as polyphenols, carotenoids and ascorbic acid (Li et al., 2014; Ma et al., 2011; Masibo and He, 2008; Schieber et al., 2000). Chlorogenic acid, gallic acid, vanillic acid, quercetin, kaempferol and mangiferin are among the major polyphenolic compounds found in mango fruit with some variations in the concentrations influenced by cultivars and different pre- and postharvest practices (Berardini et al., 2005a; Kim et al., 2007; Masibo and He, 2008; Palafox-Carlos et al., 2012a).

Mango fruit is also rich in lupeol, a naturally occurring pentacyclic triterpene renowned for its health benefits (Gallo and Sarachine, 2009; Saleem, 2009; Siddique and Saleem, 2011). The ability of lupeol in selective destruction of cancerous cells at its therapeutic doses is noteworthy (Saleem, 2009). It is also well known for its capacity to interact with multiple molecular targets in controlling carcinogenesis (Gallo and Sarachine, 2009). Lupeol could be developed as a potential agent to treat prostate cancer in humans as it is an effective inhibitor of androgen receptor *in vitro* and *in vivo* (Saleem et al., 2009).

Mangiferin (C-2-β-D-glucopyranosyl-1, 3, 6, 7-tetrahydroxyxanthone) a glucosyl xanthone known for its outstanding health benefits is another important compound present in mango fruit (Masibo and He, 2009). Mangiferin has been found to exhibit a wide range of pharmacological properties such as: antioxidant, anticancer, antimicrobial, anti-atherosclerotic, antiallergenic, anti-inflammatory and analgesic (Masibo and He, 2009).

With the increasing popularity of fruit and vegetable rich diets, there is a common concern among the consumers regarding the best stage of ripeness/maturity of these commodities that could fulfill the maximum health benefits. Due to its climacteric nature, significant physical, physiological and biochemical changes that affect the colour, firmness, taste, flavour and aroma of ripe fruit are experienced by mango during the period of ripening (Singh et al., 2013). In addition to these, several changes in the concentrations of polyphenolic compounds followed by the climacteric respiratory peak have also been reported in this climacteric fruit (Talcott et al., 2005).

The ripening-related changes in the levels of ethylene production, rate of respiration, colour, firmness, soluble solids concentration, acidity and aroma volatile production in mango fruit have been extensively studied (Ibarra-Garza et al., 2015; Lalel et al., 2003c). Besides, the effect of harvest maturity on the concentrations of some phenolic compounds, total antioxidant capacity and the concentration of total phenols in the ripe mango fruit of cultivar 'Ataulfo' (Palafox-Carlos et al., 2012a) and cultivar 'Irwin' (Kim et al., 2010a) have also been reported. The effect of fruit ripening in the concentrations of lupeol and mangiferin in Indian mangoes (Srivastava et al., 2015) and the effect of harvest maturity on the concentrations of lupeol and mangiferin in 'Ataulfo' mangoes (Ruiz-Montañez et al., 2014) were recently reported. However, the dynamics of individual and total polyphenols, lupeol, and mangiferin in the pulp and peel of mango fruit during postharvest ripening in economically important Australian mango cultivars are unknown. 'Kensington Pride' and 'R2E2' are among the prominent mango cultivars grown in Australia with uniquely low ethylene production and high sensitivity to ethylene (Lalel et al., 2003c).

We hypothesized that the concentrations of various health-promoting compounds could influence by the stages of postharvest ripening in mango fruit. Thus, the aim of the present investigation was to examine the changes in the concentrations of some important health-promoting compounds in pulp and peel of the mango fruit during postharvest ripening to determine the stage of ripening with the highest concentration of these compounds. The result of this study may identify the optimal ripe stage for

direct consumption of mango pulp or extraction of the peel with highest levels of health-promoting compounds.

6.2 Materials and methods

Detailed analytical methos and conditions can be found in Chapter 3 (page 39).

Two independent experiments were conducted to investigate the changes in the concentrations of health-promoting compounds during fruit ripening period in 'Kensington Pride' and 'R2E2' mango in 2015 and 2016 respectively.

6.2.1 Experiments

6.2.1.1 Experiment 1: Changes in the concentrations of health-promoting compounds in 'Kensington Pride' mango during postharvest ripening (2015)

Fruit, experimental design and observations recorded

Mature hard green (firmness: 162.5 N, light cream pulp, the rate of respiration: 0.19 µmol CO_2 kg^{-1} s^{-1}) 'Kensington Pride' mango fruit were sourced from a commercial orchard in Gingin (31° 27´S, 115° 55´E), Western Australia on the 4th of March 2015. The mangoes were washed with chlorinated water and air dried before transporting to the Horticulture Research Laboratory, Curtin University, Perth, Western Australia. Uniform size fruit, free from any form of visible deformities, damages or diseases were packed into soft board trays and transported to the laboratory within 2 h. The fruit were allowed to ripen at room temperature (21±1.5 °C) until eating soft stage (yellow pulp, > 75% yellow peel). Changes in the concentrations of ascorbic acid, total carotenoids, antioxidants, lupeol, mangiferin and total and individual phenols were determined in pulp and/or peel at two-day intervals during the ripening period from the date of harvesting until day 10, when the fruit is slightly over soft. The rate of respiration and ethylene production were recorded daily. Representative pulp and peel samples were prepared as described in Chapter 3, Section 3.2 and both pulp and peel samples were immediately stored at

-80 °C for the analysis of aforesaid parameters. Freeze dried (Telstar Cryodos V 1.0, Terrassa, Spain) and powdered samples of pulp and peel were used to analyze lupeol, mangiferin and phenolic acids. All the other compounds were analyzed in thawed pulp and peel. The experiment was laid out by following a completely randomized design (CRD) with four replications. Ten fruit were included in each replication.

6.2.1.2 Experiment 2: Changes in the concentrations of health- promoting compounds in 'R2E2' mango during postharvest ripening (2016)

Freshly harvested mature hard green 'R2E2' mangoes (firmness: 285.7 N, light creamy pulp, respiration rate: 0.134 μmol CO_2 kg^{-1} s^{-1}) were obtained from a commercial grower at Dongara (29° 25´S, 114° 93´E), Western Australia on the 10th of February 2016. Selection of fruit, sample preparation, observations recorded except ethylene production and the experimental design were similar to Experiment 1.

Chemicals

All the reagents and the standards of phenoli acids, lupeol and mangiferin were purchased from Sigma Aldrich (St. Louis, MO, USA) except acetonitrile, n-hexane, and methanol (Thermo Fisher Scientific, Taren Point, NSW, Australia). All the reagents used were of HPLC grade.

6.2.2 Determination of ethylene production during fruit ripening

The production of ethylene was determined using a laser-based photoacoustic ethylene detector (ETD-300, Sensor Sense, Nijmegen, The Netherlands) as explained earlier by Bulens et al. (2014) with slight modifications as described in Chapter 3, Section 3.6 and expressed in pmol kg^{-1} s^{-1}.

6.2.3 Estimation of respiration rates during fruit ripening

The rate of respiration was estimated using the infrared gas analyzer (Servomex Gas Analyzer, series 1400, East Sussex, UK) interfaced with Powerchrome 2 software as described in Chapter 3, Section 3.7. The respiration rate was calculated using the sample area and standard area of the peaks obtained from the infrared gas analyzer and expressed in μmol kg^{-1} s^{-1}.

6.2.4 Determination of health –promoting compounds

6.2.4.1 Determination of ascorbic acid

The concentration of ascorbic acid was quantified using the method described in Chapter 3, Section 3.5.5. Then the ascorbic acid concentration was calculated using a standard curve of L-ascorbic acid and was expressed as mg kg^{-1} fresh weight basis.

6.2.4.2 Estimation of total carotenoids

The total carotenoid concentration of ripe mango pulp was determined by the method described by Lalel et al. (2003a) as detailed in Chapter 3, Section 3.5.6. The total carotenoid content was expressed as mg kg^{-1} fresh weight basis.

6.2.4.3 Estimation of total antioxidants

The total antioxidant capacity of ripe mango pulp and peel was determined using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay as detailed in Chapter 3, Section 3.5.4. The total antioxidant capacity was estimated using a standard curve of Trolox (6-hydroxy-2, 5, 7, 8-tetramethychromane-2-carboxylic acid) and was expressed as mmol Trolox Equivalent Antioxidant Capacity (TEAC) kg^{-1} fresh weight basis.

6.2.4.4 Determination of lupeol

Lupeol was extracted from mango pulp/peel using the method described earlier by Ruiz-Montañez et al. (2014) with some modifications as detailed in Chapter 3, Section 3.5.1. The levels of lupeol in mango pulp/peel were determined using the method described by Oliveira et al. (2012) with some modifications as described in the same section (3.5.1). Lupeol was identified based on the retention time of the standard and spiking and quantified using lupeol standard curve. The amount was expressed as mg kg^{-1} dry weight basis.

6.2.4.5 Estimation of total phenols

The total phenol concentration was quantified using the method described previously by Robles-Sànchez et al. (2009) using Folin-Ciocalteu reagent with slight modifications as described in Chapter 3, Section 3.5.3. The total phenolic concentration was calculated using a gallic acid standard curve and expressed in g gallic acid equivalents (GAE) kg^{-1} fresh weight basis.

6.2.4.6 Determination of mangiferin and phenolic acids

The polyphenol profile in the pulp and peel of mango fruit was determined using the method described previously by Palafox-Carlos et al. (2012a) with some modifications as described in Chapter 3, Section 3.5.2. Mangiferin and the individual phenolic acids were identified using the retention times of standards and spiking. The concentrations of individual polyphenols were quantified using the standard curves of gallic acid, chlorogenic acid, vanillic acid, ferulic acid, caffeic acid and mangiferin and expressed as mg kg^{-1} dry weight basis.

6.2.5 Statistical analysis

The data were subjected to one-way ANOVA using GenStat version 14.0 (Lawes Agricultural Trust, Rothamsted, UK) software. Duncan multiple comparison test was used to calculate the significant differences. The results were recorded as means ±

standard deviation of the mean (SD). Least significant difference (LSD) was calculated following significant ($P \leq 0.05$) F-test.

6.3 Results and discussion

6.3.1 Changes in the rate of ethylene production and respiration during fruit ripening

The climacteric ethylene production peak (0.0071 pmol kg^{-1} s^{-1}) and respiration peak (0.28 μmol kg^{-1} s^{-1}) were noted on day three during fruit ripening period at 21±1 °C in cultivar 'Kensington Pride' (Fig. 6.1A). The climacteric respiration peak (0.19 μmol kg^{-1} s^{-1}) was also observed on day three of fruit ripening period in cultivar R2E2' (Fig. 6.1B). The changes in climacteric ethylene and respiration production rates observed in this study were similar to those previously reported by Lalel et al. (2003c) for 'Kensington Pride' mangoes.

6.3.2 Changes in the concentrations of health-promoting compounds during fruit ripening

6.3.2.1 Ascorbic acid

The concentrations of ascorbic acid in the pulp decreased significantly ($P \leq 0.05$) with the ripening of mango fruit. It ranged from 492.0 to 262.8 mg kg^{-1} in cultivar 'Kensington Pride' and 381.1 to 133.0 mg kg^{-1} in 'R2E2' during zero to ten days of ripening period (Fig. 6.2A). Possibly, this reduction could be ascribed to its usage as a substrate in the respiration process (Jacobi et al., 2000).

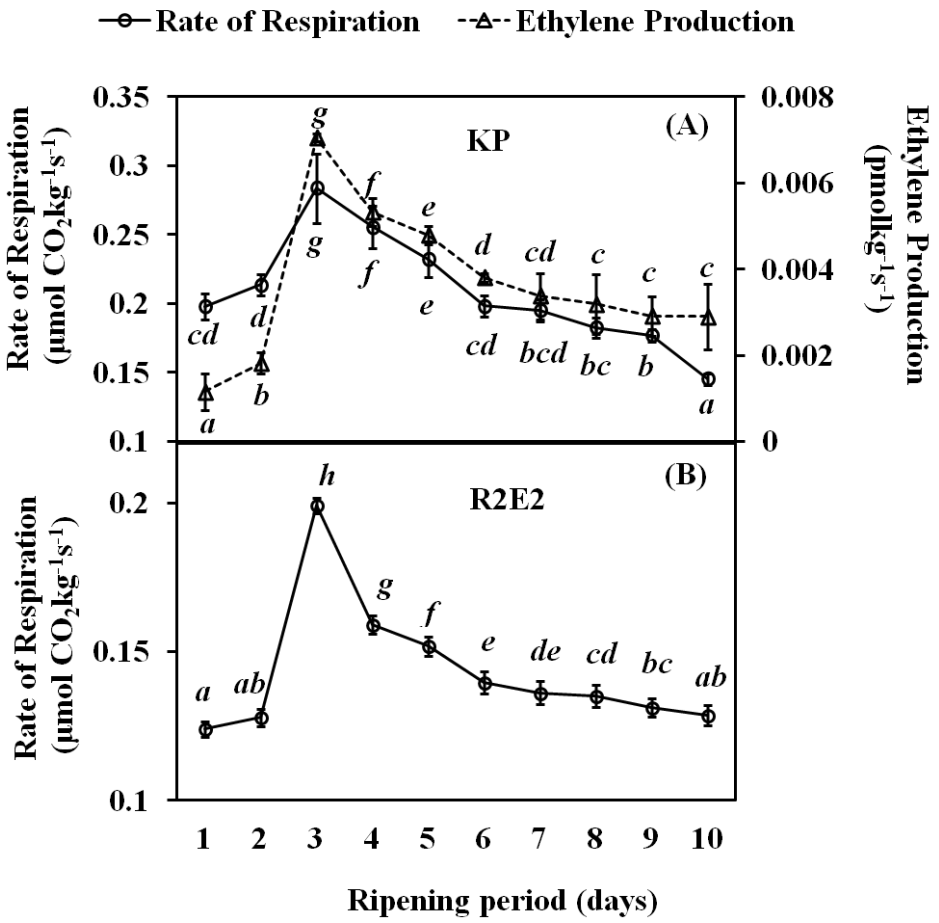

Fig. 6.1 (A-B). The rate of respiration and ethylene production in cv. 'Kensington Pride' (KP) (A) and the rate of respiration in cv. R2E2 (B) during the period of fruit ripening. n = 4 replicates in KP and n = 3 replicates in 'R2E2', 10 fruit per replication. Vertical bars represent SD of means. Mean values significantly different at $P \leq 0.05$ are indicated by different letters. Means followed by the same letter are not significantly different by Duncan multiple comparison test at $P \leq 0.05$. The lettering of the rate of respiration and ethylene production are independent from each other.

6.3.2.2 Total carotenoids

In agreement with the previous reports, (Li et al., 2014; Mercadante and Rodriguez-Amaya, 1998) a significant ($P \leq 0.05$) increase in the concentration of total carotenoids in mango pulp was observed with the advancement of fruit ripening in both the cultivars (Fig. 6.2B). This increase is considered as a well-established indication of mango fruit ripening (Mercadante and Rodriguez-Amaya, 1998). Significantly higher concentrations of total carotenoids were noted in cultivar 'Kensington Pride' on day 8 (50.6 mg kg^{-1}) and day 10 (50.9 mg kg^{-1}) and in 'R2E2' on day 10 (21.9 mg kg^{-1}) during ripening (Fig. 6.2B). A sharp increase in total carotenoid concentration was observed in 'Kensington Pride' after the 4^{th} day and it then stabilized after day 6. A significant increase in the concentration of total carotenoids was also observed in 'R2E2' mango fruit on day 4. This increase in carotenoid concentration was in parallel to the ethylene and respiratory peaks. Saltveit (1999) also reported that the elevated production of carotenoids in mango and other climacteric fruit during ripening is synchronized with the respiration and ethylene climaxes.

6.3.2.3 Total antioxidant capacity

Mango fruit is rich in antioxidants (Schieber et al., 2000) even though significant heritable differences were observed in the levels of total antioxidant capacity among different mango cultivars (Ma et al., 2011). In agreement with the findings of Palafox-Carlos et al. (2012b) the total antioxidant capacity of both pulp and peel in the present study increased significantly ($P \leq 0.05$) with ripening, irrespective of the cultivar (Fig. 6.2C and Fig. 6.2D). Possibly, it may be ascribed to a combined effect of elevated levels of carotenoids (Li et al., 2014) and polyphenols during fruit ripening (Martínez et al., 2012) as carotenoids, ascorbic acid and polyphenols are considered as the key contributors of the total antioxidant capacity of mangoes (Ma et al., 2011; Talcott et al., 2005). The total antioxidant capacity of pulp was significantly higher on day 4 (3.7 mmol TEAC kg^{-1} and 2.7 mmol TEAC kg^{-1}) and day 6 (3.8 mmol TEAC kg^{-1} and 2.8 mmol TEAC kg^{-1}) in cultivar 'Kensington Pride' and 'R2E2' respectively. After day 6, a gradual decline in the total antioxidant

capacity was observed in the pulp of both cultivars (Fig. 6.2C). This decline could be a result of the decreased concentrations of ascorbic acid following the respiration climax.

In agreement with previous reports (Ajila et al., 2007; Dorta et al., 2012), the peel of both 'Kensington Pride' and 'R2E2' mangoes showed a higher antioxidant capacity than pulp. A significantly higher capacity was noted in the peels of cultivar 'Kensington Pride' on day 8 (37.6 mmol TEAC kg^{-1}) and day 10 (37.3 mmol TEAC kg^{-1}) and in 'R2E2' (32.3 mmol TEAC kg^{-1}) on day 8 of fruit ripening (Fig. 6.2D). The total antioxidant capacity in the peel was approximately ten-fold higher than that of pulp in both cultivars. Unlike in pulp, the peel antioxidant capacity remained higher until the tenth day of fruit ripening.

6.3.2.4 Total phenols

The concentration of total phenols in the pulp of cultivar 'Kensington Pride' fruit showed a significant increase ($P \leq 0.05$) over the period of ripening from 3.0 to 5.0 g GAE kg^{-1} (Fig. 6.2E). This trend of change in total polyphenols was in agreement with previous reports (Palafox-Carlos et al., 2012b). Even though the changes in the concentrations were significant, a specific trend was not observed in 'R2E2', where the total phenols concentration ranged from 1.0 g GAE kg^{-1} to 4.0 g GAE kg^{-1} (Fig. 6.2E). A gradual decrease in the levels of total phenols in the pulp was observed in both cultivars after day 6 of fruit ripening. This decline in the level of total phenols after day 6 may indicate the reduction of phenolic acids due to utilization as respiratory substrates especially at the respiratory climax (Robles-Sánchez et al., 2009).

The total phenol concentration in the peel of both cultivars significantly ($P \leq 0.05$) increased during the period of fruit ripening (Fig. 6.2F). The highest concentrations of total phenols in the peel of 'Kensington Pride' mango fruit was found on day 8 (175.0 g GAE kg^{-1}) and day 10 (160.1 g GAE kg^{-1}) of fruit ripening and on day 10 in 'R2E2' (32.0 g GAE kg^{-1}). Mango peel is considered as a major location of polyphenols within the whole fruit (Berardini et al., 2005b; Masibo and He, 2009). In

agreement with the previous report by Kim et al. (2010a) the concentrations of total phenols in the peel were 36 and 8-folds higher than pulp in 'Kensington Pride' and 'R2E2' mangoes respectively. Significant changes in the levels of polyphenols occur in climacteric fruit such as apple, banana, and tomato due to enhanced activity of shikimic acid pathway as a result of respiratory climax (Talcott et al., 2005). The increased level of total phenols with ripening had been linked to different theories. Ribeiro et al. (2008) stated that biosynthesis of secondary metabolites such as polyphenols is induced during ripening as pathogen defence. On the other hand, Gil et al. (2000) focused on enhanced enzymatic activity and hydrolysis of complex compounds into free soluble sugars and acids as the reason for this increase. It has also been argued that polyphenol biosynthesis is induced during ripening to defend the oxidative stress generated during respiratory climax due to the production of free radicals (Masibo and He, 2008; Saltveit, 1999). Possibly, a combination of all these processes may be attributed to the ultimate increase in the level of total phenols during mango fruit ripening.

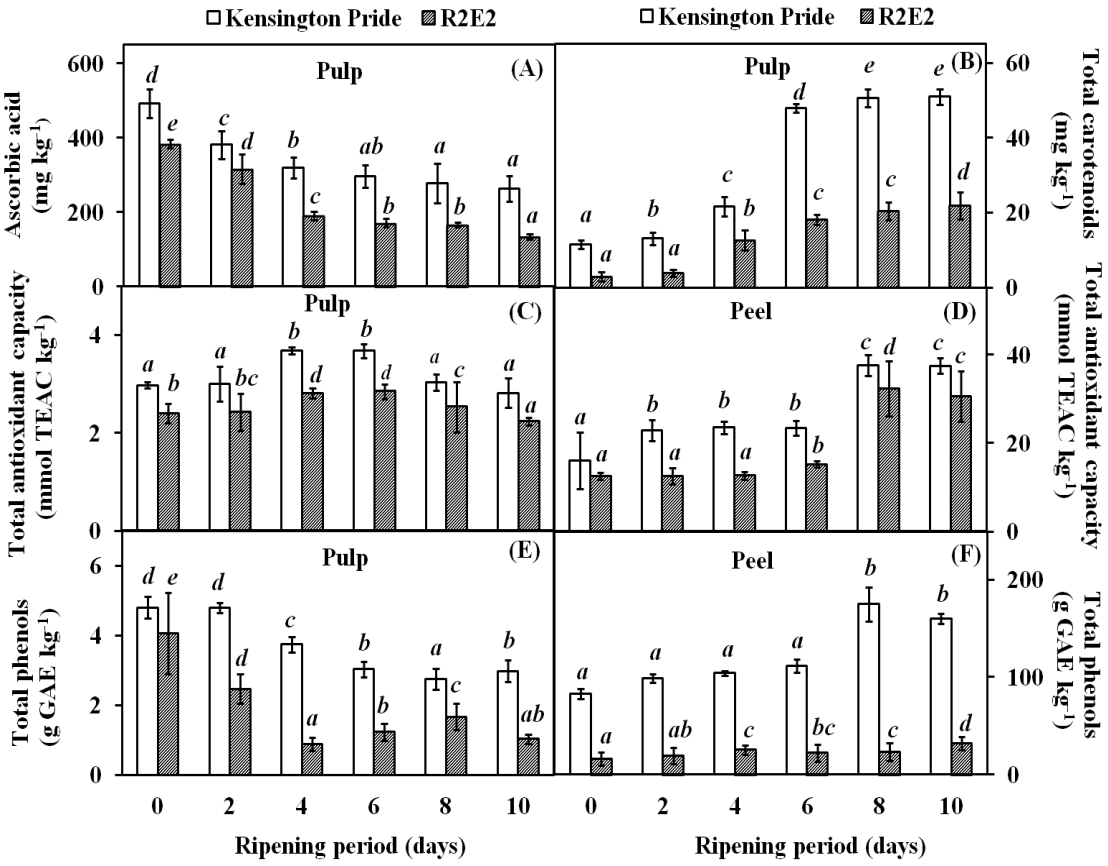

Fig. 6.2 (A-F). The concentrations of ascorbic acid in pulp (A), total carotenoids in pulp (B), total antioxidant capacity of pulp (C) and peel (D) and total phenols in pulp (E) and peel (F): cv. 'Kensington Pride' (KP) and cv. 'R2E2' during fruit ripening period. n = 4 replicates in KP and n = 3 replicates in 'R2E2', 10 fruit per replication. Vertical bars represent SD of means. Mean values significantly different at $P \leq 0.05$ are indicated by different letters. Means followed by the same letter are not significantly different by Duncan multiple comparison test at $P \leq 0.05$. The lettering of cv. 'Kensington Pride' and cv. 'R2E2' are independent from each other.

6.3.2.5 Lupeol

The concentration of lupeol was significantly affected ($P \leq 0.05$) by the process of fruit ripening (Fig. 6.3A and 6.3B). In agreement with previous reports, the highest level of lupeol was reported at the consumption maturity stage with considerable genotypic differences in the quantities (Srivastava et al., 2015; Ruiz-Montañez et al., 2014). Lupeol was not present at a detectable concentration in the pulp of cultivar 'Kensington Pride' until day 4 during the ripening period. However, concentrations of lupeol increased to 3.6 mg kg^{-1} on the sixth day and remained stable until day ten (4.1 mg kg^{-1}) (Fig. 6.3A). However, lupeol was recorded in the pulp of 'R2E2' mangoes from the date of harvesting until day 10 during the period of fruit ripening (Fig. 6.3A). Lupeol was the highest (8.8 mg kg^{-1}) on day 2, and then slightly declined until day 10 showing a significant change in the concentrations during the period of ripening ($P \leq 0.05$). These results indicate that the concentration of lupeol in the pulp and peel of mango fruit is cultivar dependent. Similar observations were recorded by Srivastava et al. (2015) for 'Bombay green', 'Dashehari', 'Langra' and 'Chausa' mangoes and Ruiz-Montañez et al. (2014) for 'Ataulfo' and autochthonous mango fruit. Unlike the pulp, lupeol was present in the peel of cultivar 'Kensington Pride' mangoes at detectable levels from the date of harvesting (Fig. 3B). Significantly higher concentrations of lupeol were observed in the peel of cultivar 'Kensington Pride' on day 2 (15.4 mg kg^{-1}) and day 4 (16.4 mg kg^{-1}) and in 'R2E2' on the fourth day (12.4 mg kg^{-1}) of ripening. This was followed by a sharp decrease in 'Kensington Pride' while a considerable level of lupeol was maintained in the peels of 'R2E2' mangoes until the eighth day of ripening (Fig. 6.3B). The changes in the concentrations of lupeol with the advancement of fruit ripening were significant ($P \leq 0.05$). The stage of ripening during which, the highest concentration of lupeol present in mango pulp and peel is also apparently cultivar specific. The highest concentration of lupeol in the pulp of 'Dashehari' and 'Chausa' mangoes were noted on day 4, whereas it was noted on day 2 and day 1 in 'Bombay Green' and 'Langra' mangoes respectively (Srivastava et al., 2015). The highest concentrations of lupeol in peel were noted on day 8 in 'Dashehari', and 'Chausa' mangoes, whereas it was noted on day 2 in 'Langra' mango (Srivastava et al., 2015). Furthermore, as previously reported by Srivastava et al. (2015) and Ruiz-Montañez et al. (2014) the peels

showed a higher level of lupeol than pulp in both cultivars. The levels of lupeol were approximately 4 and 1.4 times higher in the peel than the pulp in 'Kensington Pride' and 'R2E2' respectively. Similarly, the concentration of lupeol in 'Ataulfo' mango was 1-4 times more in the peel than pulp (Ruiz-Montañez et al., 2014), whilst it was 1-40 times more in North Indian mangoes depending on the cultivar (Srivastava et al., 2015).

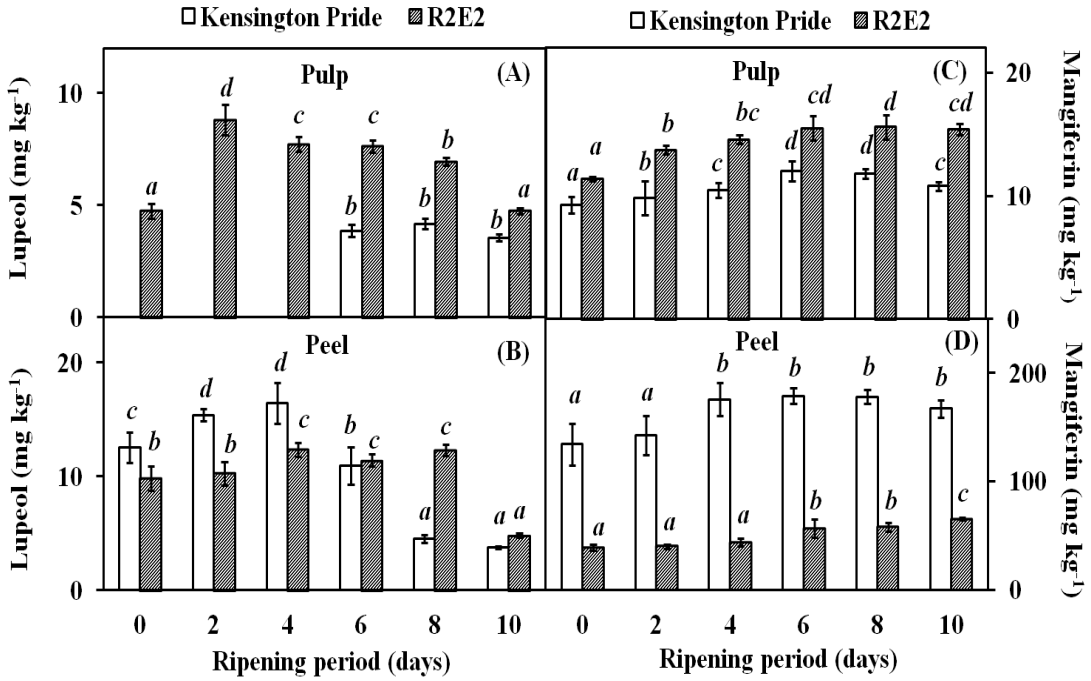

Fig. 6.3 (A-D). The concentration of lupeol in pulp (A) and peel (B) and mangiferin in pulp (C) and peel (D): cv. 'Kensington Pride' (KP) and cv. 'R2E2' during fruit ripening period. n = 4 replicates in KP and n = 3 replicates in 'R2E2', 10 fruit per replication. Vertical bars represent SD of means. Mean values significantly different at $P \leq 0.05$ are indicated by different letters. Means followed by the same letter are not significantly different by Duncan multiple comparison test at $P \leq 0.05$. The lettering of cv. 'Kensington Pride' and cv. 'R2E2' are independent from each other.

6.3.2.6 Mangiferin

The concentration of mangiferin in pulp and peel significantly increased during the process of ripening ($P \leq 0.05$) (Fig. 6.3C and 6.3D). Significantly higher concentrations of mangiferin in the pulp of ripe fruit were noted on day 6 (12.0 mg kg^{-1}) and day 8 (11.8 mg kg^{-1}) in cultivar 'Kensington Pride' while it was recorded (16.1 mg kg^{-1}) on day 8 of ripening in 'R2E2' (Fig. 6.3C). A similar trend of changes in the concentration of mangiferin in the pulp was reported earlier in 'Bombay green' and 'Langra' mango fruit during ripening (Srivastava et al., 2015). The concentration of mangiferin was significantly increased after day 2 in the peel in 'Kensington Pride' and from day 4 in 'R2E2' until day 10 (Fig. 6.3D). However, the concentration of mangiferin in the peel of 'Langra' mango fruit significantly decreased over the period of ripening showing the highest concentration on day 2, whereas it increased with ripening in 'Dashehari' and 'Chausa' mangoes (Srivastava et al., 2015). Thus, the changes in the concentration of mangiferin in the pulp and peel of mango fruit are apparently cultivar dependent. The peels exhibited approximately 15 and 5 times higher concentrations of mangiferin than the pulp over the entire period of ripening in 'Kensington Pride' and 'R2E2' respectively. Previously, Manthey and Perkins-Veazie (2009) reported that the amount of mangiferin was low in the pulp of five major mango cultivars: 'Tommy Atkins', 'Kent', 'Keitt', 'Haden' and 'Ataulfo'. According to Berardini et al. (2005a), the concentrations of mangiferin in the pulp and peel of mature mangoes of cultivar 'R2E2' were 4.3 mg $kg^{-1}$and 89.2 mg kg^{-1} respectively which are comparable with our results. However, Daud et al. (2010) found that the level of mangiferin in the pulp of ripe 'R2E2' mangoes was not at a detectable level, while trace amounts were present in the peel. Moreover, significant inter-fruit variations in the amount of mangiferin in the same cultivar had been observed by them in addition to genotypic effects. Hewavitharana et al. (2013) quantified the inter-fruit difference in the amount of mangiferin in 'Kensington Pride' mango as 139%. Thus, it is rather difficult to generalize the amounts of mangiferin in mango pulp. Nevertheless, in the present study, the level of mangiferin in the peel was notably superior to the level in pulp irrespective of the cultivar in accordance with previous reports (Ruiz-Montañez et al., 2014).

6.3.2.7 Phenolic acids

The concentrations of polyphenolic compounds in mango pulp and peel changed significantly with ripening ($P \leq 0.05$). Polyphenols arranged in relative abundance showed that gallic acid, chlorogenic acid, and vanillic acid were the major polyphenols present in pulp and peel of mango fruit (Fig. 6.4 A-C and Fig. 6.4 F-H). Gallic acid has previously been reported as the major polyphenol in the pulp of other mango cultivars (Masibo and He, 2008; Talcott et al., 2005). In addition to these phenolic compounds, ferulic acid (Fig. 6.4D) and caffeic acid (Fig. 6.4E) were also present in mango pulp in comparatively lower concentrations. Quercetin was not detected in the pulp or peel of 'Kensington Pride' and 'R2E2' mango fruit during the whole ripening period. Similarly, Berardini et al. (2005a) and Pierson et al. (2014) also reported that quercetin was not in quantifiable concentrations in cultivar 'R2E2' and 'Kensington Pride'. The concentration of gallic acid in the pulp of 'Kensington Pride' ranged from 99.6 to 363.7 mg kg^{-1} (Fig. 6.4A), while chlorogenic acid (Fig. 6.4B) and vanillic acid (Fig. 6.4C) ranged from 98.3 to 200.7 mg kg^{-1} and 27.0 to 111.7 mg kg^{-1} respectively. The concentrations of gallic acid, chlorogenic acid and vanillic acid in the pulp of 'R2E2' mango fruit ranged from 161.9 to 222.9 mg kg^{-1}, 4.8 to 22.5 mg kg^{-1} and 9.6 to 20.2 mg kg^{-1} respectively (Fig. 6.4A-C). The concentrations of ferulic acid and caffeic acid changed significantly over the progression of fruit ripening at $P \leq 0.05$ level. Significantly higher concentrations of ferulic acid in the pulp of 'Kensington Pride' were recorded on day 8 (6.9 mg kg^{-1}) and day 10 (6.4 mg kg^{-1}) and in 'R2E2' from day 6 to 10 during ripening (Fig. 6.4D), whilst, significantly higher concentrations of caffeic acid were noted from day 2 to day 6 (2.6 – 2.7 mg kg^{-1}) in the pulp of 'Kensington Pride' and from day 6 to day 8 (2.2 – 2.4 mg kg^{-1}) in the pulp of 'R2E2' mangoes (Fig. 6.4E). The highest concentrations of gallic, chlorogenic and vanillic acids in the peel of 'Kensington Pride' and 'R2E2' mango fruit were found during the post-climacteric ripening stage, (Fig. 6.4F-H). The highest concentration of ferulic acid in the peels of cultivar 'Kensington Pride' (41.2 mg kg^{-1}) and cultivar 'R2E2 (20.2 mg kg^{-1}) were observed on day 6 and day 10 respectively (Fig. 6.4I). Significantly higher concentrations of caffeic acid in the peel of 'Kensington Pride' mango fruit were observed on day 2 (19.1 mg kg^{-1}) and day 4 (20.2 mg kg^{-1}), whilst the concentrations of caffeic acid

were significantly high on day 6 (14.1 mg kg^{-1}) and day 8 (14.0 mg kg^{-1}) in the peel of 'R2E2' mango fruit (Fig. 6.4J). The concentrations of gallic acid, chlorogenic acid, vanillic acid, ferulic acid and caffeic acid in the peel of 'Kensington Pride' mango were approximately 20, 2, 10, 6, and 7 folds higher than the pulp respectively, while these levels were 25, 3, 20, 30 and 5 folds higher in the peels of 'R2E2' mango than pulp respectively. The polyphenol profiles found in the pulp and peel in both cultivars were in accordance with the previously reported (Masibo and He, 2008; Palafox-Carlos et al., 2012a).

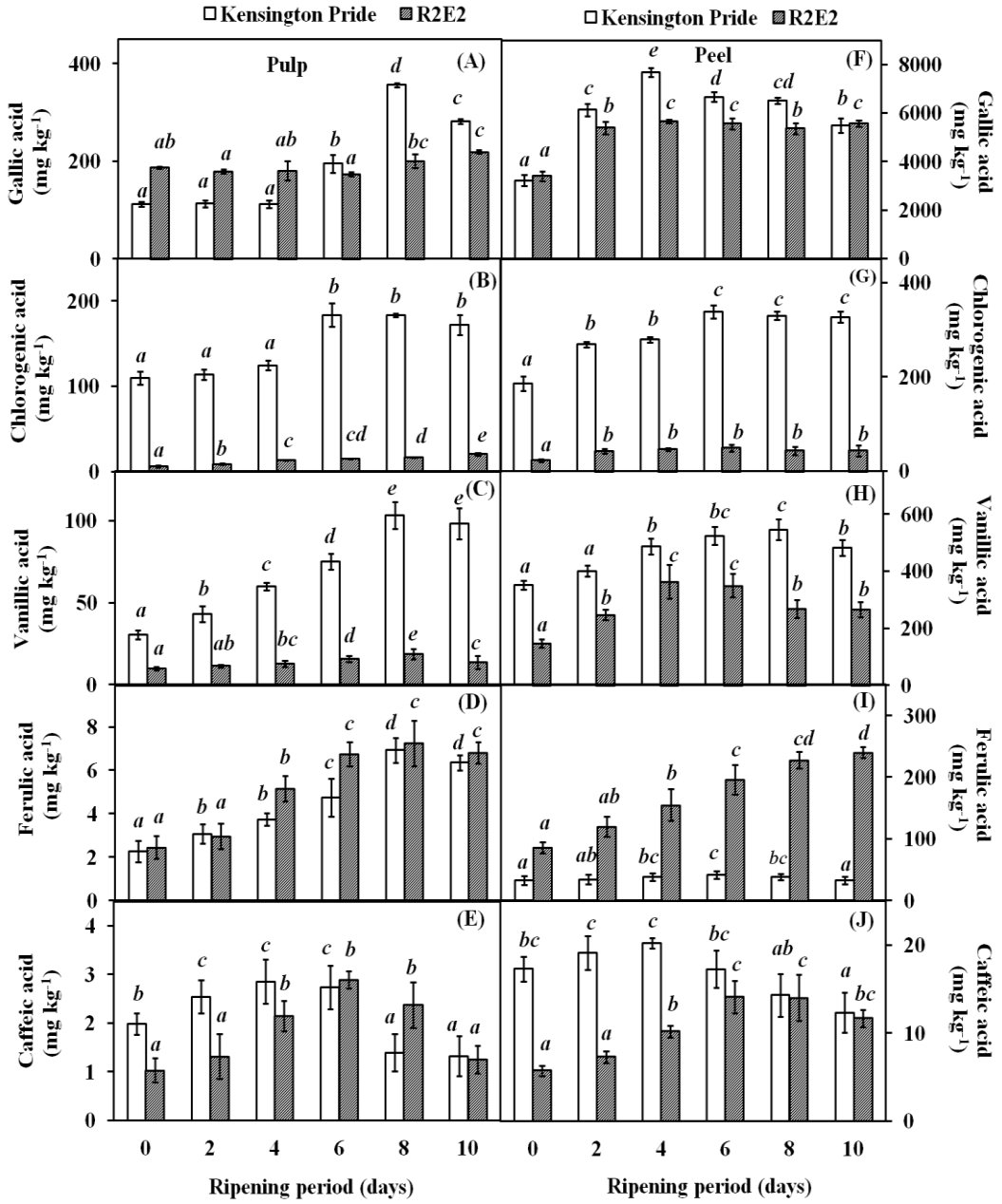

Fig. 6.4 (A-J). The concentrations of phenolic acids in pulp (A-E) and peel (F-J): cv. 'Kensington Pride' (KP) and cv. 'R2E2' during fruit ripening period. n = 4 replicates in KP and n = 3 replicates in 'R2E2', 10 fruit per replication. Vertical bars represent SD of means. Mean values significantly different at $P \leq 0.05$ are indicated by different letters. Means followed by the same letter are not significantly different by Duncan multiple comparison test at $P \leq 0.05$. The lettering of cv. 'Kensington Pride' and cv. 'R2E2' are independent from each other.

6.4 Conclusion

The concentrations of health-promoting compounds present in mango pulp and peel were significantly affected by the process of fruit ripening in the present study. The concentrations of total carotenoids, total antioxidant capacity in peel and pulp, total phenols in the peel, and mangiferin were elevated during the post-climacteric phase. On the other hand, the concentration of ascorbic acid declined with ripening. The concentration of lupeol, in the pulp of cv. 'Kensington Pride' was also on the rise during ripening, while its level did not follow a clear trend in cv. 'R2E2'. Gallic acid, chlorogenic acid, and vanillic acid were the major polyphenolic compounds identified in both pulp and peel of studied cultivars at all stages of ripening. Ferulic acid and caffeic acid were present in lower amounts in the pulp and peel of both cultivars. Overall, mangoes at post-climacteric ripening phase could offer the highest concentrations of health-promoting compounds. Peels at this stage of ripening could be used as a good source to extract these compounds. However, cellular antioxidant assays to confirm the bioactivity of mango extracts warrant investigation. Additionally, in-depth analysis of polyphenolic compounds using HPLC-MS would further the knowledge of potential health benefits of mango fruit.

CHAPTER 7

Cold storage temperatures and durations affect the concentrations of lupeol, mangiferin, phenolic acids and other health-promoting compounds in the pulp and peel of ripe mango fruit

ABSTRACT

Mangoes are usually stored above 13 ℃ to avoid chilling injury. We investigated the effects of cold storage temperatures (5 and 13 ℃) and durations (12 and 24 d) on the concentrations of lupeol, mangiferin, phenolic acids (gallic, chlorogenic, vanillic, ferulic and caffeic), ascorbic acid, carotenoids, total phenols and antioxidants in the pulp and peel of ripe 'Kensington Pride' mango fruit. Mature green mangoes were stored at 5 ℃ (chilling) or 13 ℃ (non-chilling) temperature for 12 and 24 d prior to ripening at ambient temperature (21 ± 1.5 °C). Chilling injury and concentrations of health-promoting compounds were determined at eating soft ripe stage. Chilling injury symptoms were only developed on ripe fruit following storage at 5 ℃ for 24 d. The concentrations of lupeol in pulp and peel, chlorogenic and caffeic acids in the pulp were significantly higher in fruit stored at 5 ℃ than 13 ℃, whilst mangiferin, gallic, chlorogenic, vanillic, ferulic, and caffeic acids, total phenols, antioxidants and carotenoids in the peel were significantly higher when stored at 13 ℃. The concentrations of lupeol and chlorogenic acid in pulp and peel and gallic acid in the pulp were significantly lower when stored for 24 d compared to 12 d, whilst vanillic acid, total phenols, total antioxidants and ascorbic acid in the pulp and caffeic acid in both pulp and peel were significantly higher when stored for 24 d. In conclusion, cold storage temperatures and duration influence the concentration of lupeol, mangiferin, phenolic acids and other health-promoting compounds in the pulp and

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peel of ripe mango fruit. Storage of mature green mangoes at chilling temperature (5 °C) for 12 d prior to ripening (21 ± 1.5 °C) seems to be a promising tool for maximizing the levels of lupeol in the pulp and peel of the fruit.

KEYWORDS: *mango; low-temperature storage; chilling injury; health-promoting compounds; lupeol; mangiferin; phenolic acids*

7.1 Introduction

Mango (*Mangifera indica* L.) is globally known for its appealing taste and excellent nutritional quality. Additionally, particular health-promoting compounds present in this fruit are also known for their ability to reduce the risk of chronic health issues (Masibo and He, 2008). Lupeol and mangiferin are two such compounds with a significant protective potential. Lupeol, a triterpene is one of the most important anti-carcinogenic compounds present in mango, and has been found to be capable of reducing the risk of a number of serious human diseases including cancer, cardiovascular diseases, diabetes, liver toxicity and renal diseases (Saleem, 2009; Siddique and Saleem, 2011). Mangiferin, a glucosyl xanthone is also known for its wide range of health protective properties such as antioxidant, anticancer, antimicrobial, cardio-protective and anti-inflammatory (Masibo and He, 2008). Moreover, a number of studies have revealed that the pulp, peel, seed and other parts of mango tree are good sources of health-promoting compounds including gallic acid, chlorogenic acid, vanillic acid among many other polyphenolic antioxidants which have a well-known potential in reducing the risk of cancer and cardiovascular diseases (Ajila et al., 2007; Masibo and He, 2008; Kim et al., 2010a). Mango fruit is also rich in other dietary antioxidants, such as ascorbic acid and carotenoids which contribute to its health promoting potential (Kim et al., 2007; Ma et al., 2011).

The storage life of mango fruit is extremely limited; with fruit usually ripen in a week after harvest at mature green stage at ambient temperature (Singh et al., 2013). Therefore; the mango fruit are usually stored under low temperatures to prolong storage life (Chaplin et al., 1991; Medlicott et al., 1990; Talcott et al., 2005). Cold storage technology; however, cannot be exploited to its full potential in extending

storage life of tropical and subtropical fruit including mango because of their susceptibility to chilling injury. Mango fruit when stored below 13 °C develop chilling injury symptoms (Chaplin et al., 1991). Previously, the impact of low-temperature storage on chilling injury and physico-chemical parameters such as colour, pulp firmness, soluble solids concentration, acidity and total and individual sugars of mango fruit have been reported (Chaplin et al., 1991; Nair and Singh., 2009; Robles-Sánchez et al., 2009; Sankat et al., 1994). Some limited and inconclusive research has been reported on the impact of cold storage and chilling injury on the concentrations of health-promoting compounds such as ascorbic acid, total antioxidants, total carotenoids and total phenols in mango fruit (Kondo et al., 2005; Nair and Singh., 2009; Robles-Sánchez et al., 2009). However, no research work has been reported on the effects of low temperature storage on the concentrations of potential anticancer compounds such as lupeol, mangiferin and phenolic acids including gallic acid, chlorogenic acid and vanillic acid in the pulp and peel of mango fruit.

Given the potential health benefits of polyphenols, there have been recent reports in the use of physical elicitors (low temperature storage, heat treatment, controlled and modified atmosphere storage) and chemical elicitors (methyl jasmonate, salicylic acid and ethylene) as an effective tool to trigger their production in fruit and vegetables (Ruiz-Garcia and Gomez-Plaza, 2013; Schreiner and Huyskens-Keil, 2006). The low temperature stress is believed to induce the biosynthesis of polyphenols via the shikimic acid pathway as a part of the plant defence mechanism (Ruiz-Garcia and Gomez-Plaza, 2013). Previously, Rivera-Pastrana et al. (2010) claimed an increased level of total antioxidants and better retention of ferulic acid and caffeic acid in the chill-sensitive fruit papaya; when stored at 5 °C. Therefore, the effect of cold storage temperatures and periods on the levels of phenolic compounds in ripe mango fruit warrants to be investigated as a potential tool to enhance its health beneficial properties.

In this study, it was hypothesised that the chill-storage temperature would increase the concentrations of lupeol, mangiferin and phenolic acids (chlorogenic acid, gallic acid, vanillic acid, ferulic acid and caffeic acid) and other health-promoting

compounds (ascorbic acid and carotenoids) as a response to low temperature stress. To the best of our knowledge this is the first study on the effect of chilling and non-chilling low temperature storage and period on the concentrations of lupeol, mangiferin and phenolic acids (gallic acid, chlorogenic acid, vanillic acid, ferulic acid and caffeic acid) in ripe mango fruit.

7.2 Materials and methods

All the procedures and conditions are detailed in Chapter 3 (page 39).

7.2.1 Materials

Fruit

Hard green mature 'Kensington Pride' mango fruit (light cream pulp, firmness: 165±1 N) were harvested from a commercial orchard in Gingin (31° 27´S, 115° 55´E), Western Australia and transported within 2 h to the laboratory on the 9th of March 2015. Only mango fruit free from visual symptoms of mechanical, chemical or insect-pest injuries and symptoms of disease(s) were used in the study. The selected fruit were treated with the fungicide Sportak (0.55 ml L^{-1}) containing prochloraz as the active ingredient (Bayer CropScience Pty Ltd., Victoria, Australia) to prevent disease development during storage and allowed to dry. The fruit were packed into cardboard boxes and stored at either 5 °C or 13 °C at 85% ± 0.5% relative humidity in dark for either 12 d or 24 d. After each storage period at both temperatures, the fruit were allowed to ripen at ambient temperature (21±1.5 °C) until eating soft stage (yellow pulp and >75% yellow peel, Firmness: 7.0 – 9.0 N). The experiment followed two-factor factorial design (storage temperature and storage duration). Ten mangoes were used for each treatment unit and replicated four times.

Once mangoes reached the eating soft stage, the chilling injury symptoms were recorded. The peel and pulp samples of 10 mango fruit in each replication prepared as described in Chapter 3, Section 3.2 were immediately stored at -80 °C for the later

determination of lupeol, mangiferin, phenolic acids, total phenols, total antioxidants, ascorbic acid and total carotenoids. The concentrations of total phenols, total antioxidants, ascorbic acid and total carotenoids were determined using thawed samples of each replication. Some representative frozen, samples were freeze dried as described in the same section (3.2) powdered and stored at – 20 °C for the later determination of the concentrations of lupeol, mangiferin and phenolic acids.

Chemicals

All reagents and standards of lupeol, mangiferin, phenolic acids, β-carotene, L-ascorbic acid and Trolox were purchased from Sigma Aldrich (St. Louis, MO, USA) whilst methanol, acetonitrile and n-hexane were purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Taren Point, NSW, Australia). Only HPLC grade reagents and standards were used in the study.

7.2.2 Chilling injury (CI)

The level of chilling injury (CI) on the ripe mango fruit was recorded using the following rating scale previously described by Zaharah and Singh (2011b); 0- no damage, 1- very light damage (< 5% of the surface damaged), 2- light damage (5 – 10% of the surface is damaged), 3- moderate damage (11- 24%) and 4- severe damage (25 - 50% of the surface damaged).The chilling injury index was calculated using the following formula;

$$\frac{\sum (\text{Injury level} \times \text{number of fruit at each level})}{\text{Total number of fruit}}$$

7.2.3 Determination of the concentrations of health-promoting compounds

7.2.3.1 Lupeol

Lupeol was extracted from the freeze dried mango pulp/peel and quantified using an Agilent HPLC system (1200 series, Agilent Technologies, Ratingen, Germany) fixed

with a diode array detector (1200 Infinity, Agilent Technologies) as described in Chapter 3, Section 3.5.1. The amount of lupeol was quantified using a standard curve and expressed as mg kg^{-1} dry weight basis.

7.2.3.2 Total phenols

The total phenol concentration was estimated following the method described earlier by Robles-Sànchez et al. (2009) using Folin-Ciocalteu reagent with slight modifications which have been described in Chapter 3, Section 3.5.3. A gallic acid standard curve was used to calculate the total phenol concentration and the values were expressed in g GAE kg^{-1} fresh weight basis.

7.2.3.3 Mangiferin and phenolic acids

Determination of the concentrations of mangiferin and phenolic acids (gallic, chlorogenic, vanillic, ferulic and caffeic) was carried out following the method previously described by Palafox-Carlos et al. (2012a) with few modifications and conditions of analysis that have been detailed in Chapter 3, Section 3.5.2. The concentrations of these polyphenols were quantified using standard curves of each compound and expressed as mg kg^{-1} dry weight basis.

7.2.3.4 Ascorbic acid

The method described previously by Malik and Singh (2005) detailed in Chapter 3, Section 3.5.5 was followed to determine the concentration of ascorbic acid in the fruit pulp. The amount of ascorbic acid was calculated based on an L-ascorbic acid standard curve and expressed as mg kg^{-1} fresh weight basis.

7.2.3.5 Total carotenoids

The method described in Chapter 3, Section 3.5.6 was used to determine the concentration of total carotenoids in the pulp of ripe mango fruit and the

concentration of total carotenoids was calculated using a β-carotene standard curve and expressed as mg kg^{-1} fresh weight basis.

7.2.3.6 Total antioxidants

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay as described in Chapter 3, Section 3.5.4 was used to assess the total antioxidant capacity in the pulp and peel of ripe mango fruit. The total antioxidant capacity was estimated using a standard curve of Trolox. Results were expressed as mmol Trolox equivalent antioxidant capacity (TEAC) kg^{-1} fresh weight basis.

7.2.4 Statistical analysis

Data were subjected to two-way ANOVA (low storage temperatures and storage duration). Least significant differences were calculated using Fisher's Protected Least Significant Test ($P \leq 0.05$). The effect of storage temperature, storage duration and their interaction on chilling injury and the concentrations of health-promoting compounds were assessed. Genstat version 14.0 (Lawes Agricultural Trust, Rothamsted, UK) software was used to analyze the data.

7.3 Results

7.3.1 Chilling injury (CI)

Chilling injury symptoms were only developed in those mangoes stored at 5 °C for 24 d after ripening at room temperature (21 ± 1.5 °C). The CI index was the highest (1.8) when the fruit were stored at 5 °C for 24 d following ripening at room temperature (21 ± 1.5 °C) for 7 d when compared to all other treatments (0) (Fig. 7.1).

7.3.2 Effect of chilling and non-chilling storage temperatures and durations on concentration of health-promoting compounds

7.3.2.1 Lupeol and mangiferin

The concentration of lupeol in the pulp and peel of ripe mangoes was significantly affected by low storage temperature and duration (Table 7.1).When averaged over both storage periods; mean concentrations of lupeol were significantly higher in those fruit previously stored at 5 °C than those previously stored at 13 °C. When averaged over both storage temperatures, the mean concentrations of lupeol in both pulp and peel of ripe fruit were significantly higher in 12 d stored fruit, than those stored for 24 d.

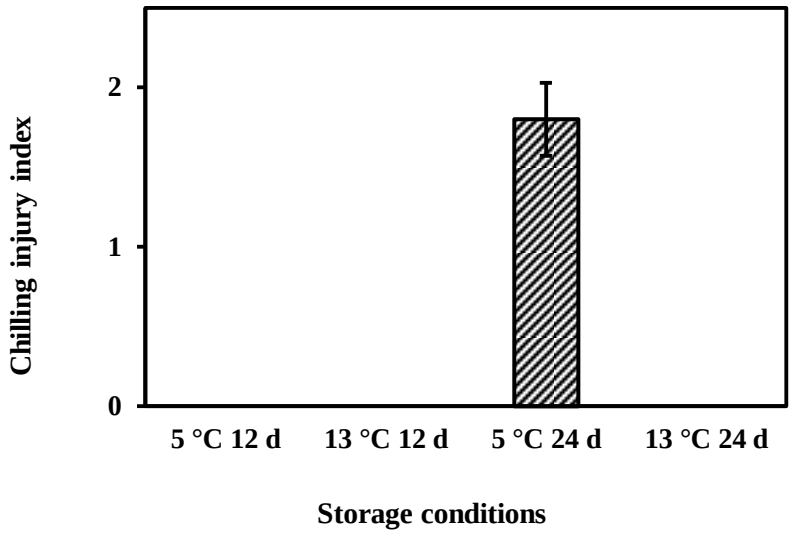

Fig. 7.1 Chilling injury index of the ripe mango fruit following cold storage at 5 °C and 13 °C for 12 d or 24 d. n = 4, 10 fruit in each replicate. The vertical bar shows the standard deviation of means.

Table 7.1. The concentrations of lupeol and mangiferin in the pulp and peel of ripe mangoes exposed to different cold storage temperatures and durations

Storage temperature	Lupeol (mg kg^{-1} DW)						Mangiferin (mg kg^{-1} DW)					
	Pulp			Peel			Pulp			Peel		
	Storage period (d)			Storage Period (d)			Storage period (d)			Storage Period (d)		
	12	24	Mean (ST)	12	24	Mean (ST)	12	24	Mean (ST)	12	24	Mean (ST)
5 °C	12.3±1.3^{d}	8.4±1.0^{b}	10.4^{A}	15.2±1.1^{b}	14.5±1.2^{b}	14.9^{A}	8.8±0.1^{a}	9.2±0.2^{a}	9.0	11.9±0.6^{bc}	10.0±0.3^{a}	10.9^{B}
13 °C	10.8±0.7^{c}	3.7±0.2^{a}	7.2^{B}	15.3±1.2^{b}	4.1±0.1^{a}	9.7^{B}	12.2±2.3^{b}	8.9±0.1^{a}	10.6	11.3±0.7^{b}	12.5±0.4^{c}	11.9^{A}
Mean (SP)	11.6^{A}	6.0^{B}		15.3^{A}	9.3^{B}		10.5	9.1		11.6	11.2	
LSD ($P \leq 0.05$)	ST	SP	ST×SP	ST	SP	ST×SP	ST	SP	ST×SP	ST	SP	ST×SP
	1.086	1.086	1.535	1.443	1.443	2.041	NS	NS	2.141	0.723	NS	1.023

DW = Dry weight, ST = Storage temperature, SP = Storage period, ST×SP = Storage temperature × storage period interaction, NS = Not significant, n = 4 ± SD, 10 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by lower case, superscript letters within the comparison between storage temperature and period. Significant difference between the mean storage temperatures with each period is represented by different upper case, superscript letters. All of the lettering is separate for pulp and peel and for different compounds.

Overall, the concentration of lupeol in the peel of ripe mango fruit was 1.4 fold higher than in the pulp. The interaction between the low storage temperature and duration was significant ($P \leq 0.05$) for the concentrations of lupeol in both the pulp and peel.

The storage duration did not significantly affect the concentration of mangiferin in the pulp and peel of ripe fruit (Table 7.1). Its concentration in the pulp of ripe fruit was not affected by the storage temperature whilst, in the peel it was significantly ($P \leq 0.05$) lower in the fruit previously kept at 5 °C compared to those kept at 13 °C. The interaction between low storage temperature and duration was significant for the concentrations of mangiferin in both the pulp and peel. The pulp of the ripe mango fruit previously stored at 13 °C for 12 d and peel of those fruit stored at 13 °C for 24 d exhibited the highest concentrations of mangiferin (Table 7.1). Across all samples, the concentration of mangiferin in the peel of ripe mango fruit on average was 1.2 times higher than pulp.

7.3.2.2 Phenolic acids

Gallic, chlorogenic, vanillic, ferulic and caffeic acids were identified in the pulp and peel of all ripe mangoes previously stored at chilling (5 °C) and non-chilling (13 °C) temperatures for 12 or 24 d (Table 7.2). Their concentrations in both pulp and peel were significantly affected ($P \leq 0.05$) by the low storage temperature except for the concentration of ferulic acid in pulp. The storage duration also influenced the concentrations of all of the phenolic acids in the pulp except that of ferulic acid. The concentrations of gallic, vanillic and ferulic acids in the peel were also not significantly ($P \leq 0.05$) affected by the storage duration. The interaction between low storage temperature and duration was found to be significant ($P \leq 0.05$) for the concentrations of gallic, vanillic, ferulic and caffeic acids in the peel and the concentration of vanillic and caffeic acids in the pulp. When averaged over both storage periods, mean concentrations of gallic acid and vanillic acid in both pulp and peel and the concentrations of chlorogenic, ferulic and caffeic acids in peel of ripe fruit were significantly higher when stored at 13 °C compared to 5 °C (Table 7.2). When averaged over storage temperatures, the mean concentrations of gallic acid and

chlorogenic acid in the pulp and the concentration of chlorogenic acid in peel were significantly higher in 12 d cold stored fruit than those stored for 24 d. In contrast, the concentrations of vanillic and caffeic acids in the pulp of ripe fruit were significantly higher after 24 d of cold storage, irrespective of the storage temperature (Table 7.2). Overall, the concentrations of gallic acid, chlorogenic acid, vanillic acid, ferulic acid and caffeic acids in the peel of ripe mango fruit were 2.6, 1.6, 2.6, 2.6 and 16.0 times higher respectively than in the pulp.

7.3.2.3 Total phenols and total antioxidant capacity

The concentration of total phenols in the pulp was significantly affected only by the low temperature storage period ($P \leq 0.05$). The mean concentration of total phenols in the pulp was significantly higher when stored for 24 d than in those stored for 12 d, irrespective of the storage temperature (Table 7.3). Whilst, the mean concentration of total phenols in the peel was significantly higher in the fruit previously stored at 13 °C than those stored at 5 °C.

When averaged over the storage period, the mean total antioxidant capacity in the pulp was significantly higher in those fruit stored at 5 °C than those kept at 13 °C. However, the trend was reversed in the peel (Table 7.3). The mean total antioxidant capacity in the peel was significantly higher in 24 d storage than 12 d storage, irrespective of the storage temperature. Overall the total phenol concentration and total antioxidant capacity in the peel of ripe mango fruit were 22.1 and 9.7 times higher respectively than pulp.

Table 7.2. The concentrations of phenolic acids: gallic, chlorogenic, vanillic, ferulic and caffeic in the pulp and peel of ripe mangoes exposed to different cold storage temperatures and durations

Storage temperature	Gallic acid (mg kg^{-1} DW)						Chlorogenic acid (mg kg^{-1} DW)					
	Pulp			Peel			Pulp			Peel		
	Storage period (d)			Storage period (d)			Storage period (d)			Storage period (d)		
	12	24	Mean (ST)	12	24	Mean (ST)	12	24	Mean (ST)	12	24	Mean (ST)
5 °C	213.0±9.9^{bc}	75.0±10.2^{a}	144.0^{B}	213.3±11.9^{a}	207.0±48.2^{a}	210.0^{B}	174.4±42.3	129.7±8.2	152.1^{A}	242.4±21.6	121.8±16.1	182.1^{B}
13 °C	290.0±77.2^{c}	140.0±16.7^{a}	215.0^{A}	654.0±44.3^{c}	793.0±90.5^{b}	723.5^{A}	128.0±7.1	108.2±4.9	118.1^{B}	282.0±19.6	210.3±63.4	246.2^{A}
Mean (SP)	252.0^{A}	107.5^{B}		434.0	500.0		151.2^{A}	119.9^{B}		262.2^{A}	166.1^{B}	
LSD ($P \leq 0.05$)	ST	SP	ST×SP	ST	SP	ST×SP	ST	SP	ST×SP	ST	SP	ST×SP
	55.8	55.8	NS	70.9	NS	100.3	27.9	27.9	NS	49.7	49.7	NS
	Vanillic acid (mg kg^{-1} DW)						Ferulic acid (mg kg^{-1} DW)					
5 °C	77.4±7.2^{a}	81.9±7.9^{a}	79.7^{B}	198.233.7b	106.7±7.6a	152.5^{B}	16.0±1.8	17.8±4.5	16.9	35.8±7.4^{a}	28.3±3.8^{a}	32.05^{B}
13 °C	79.9±5.1^{a}	124.9±7.3^{b}	102.4^{A}	280.7±49.9c	374.9±37.4d	327.8^{A}	21.9±5.8	19.3±4.0	20.6	59.3±6.5^{b}	69.2±12.2^{b}	64.25^{A}
Mean (SP)	78.9^{B}	103.4^{A}		239.4	240.8		18.95	18.6		47.55	48.75	
LSD ($P \leq 0.05$)	ST	SP	ST×SP	ST	SP	ST×SP	ST	SP	ST×SP	ST	SP	ST×SP
	10.3	10.3	14.5	40.1	NS	56.7	NS	NS	NS	9.2	NS	13.03
	Caffeic acid (mg kg^{-1} DW)											
5 °C	1.7±0.1^{b}	2.9±0.3^{c}	2.3^{A}	19.6±2.3^{a}	25.8±4.7^{a}	22.7^{B}						
13 °C	1.1±0.4^{a}	2.2±0.5^{b}	1.65^{B}	33.7±7.2^{a}	46.9±11.1^{b}	40.3^{A}						
Mean (SP)	1.4^{B}	2.55^{A}		26.7^{B}	36.4^{A}							
LSD ($P \leq 0.05$)	ST	SP	ST×SP	ST	SP	ST×SP						
	0.5	0.5	0.7	7.8	7.8	11.03						

DW = Dry weight, ST = Storage temperature, SP = Storage period, ST×SP = Temperature × storage period interaction, NS = Not significant, n = 4 ± SD, 10 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by lower case, superscript letters within the comparison between storage temperature and period. Significant difference between the mean storage temperatures with each period is represented by different upper case, superscript letters. All of the lettering is separate for pulp and peel and for different compounds.

Table 7.3. The concentrations of total phenols and total antioxidant capacity of the pulp and peel of ripe mangoes exposed to different cold storage temperatures and durations

Storage temperature	Total phenols (g GAE kg^{-1} FW)						Total antioxidant capacity (mmol TEAC kg^{-1} FW)					
	Pulp			Peel			Pulp			Peel		
	Storage period (d)			Storage period (d)			Storage period (d)			Storage period (d)		
	12	24	Mean (ST)	12	24	Mean (ST)	12	24	Mean (ST)	12	24	Mean (ST)
5 °C	4.0±0.5	7.0±1.2	5.5	118.2±16.7^{b}	89.6±30.0^{a}	103.9^{B}	17.9±1.6^{b}	17.9±1.8^{b}	17.9^{A}	101.3±2.3^{a}	137.9±2.8^{c}	119.6^{B}
13 °C	5.0±1.1	6.2±1.5	5.6	117.0±18.4^{b}	166.2±17.9^{c}	141.6^{A}	12.2±0.7^{a}	11.5±1.5^{a}	11.8^{B}	129.0±8.5^{b}	206.7±6.9^{d}	167.8^{A}
Mean (SP)	4.5^{B}	6.6^{A}		117.6^{A}	127.9^{A}		15.0^{A}	14.7^{A}		115.1^{A}	172.3^{B}	
LSD ($P \leq 0.05$)	ST	SP	ST×SP	ST	SP	ST×SP	ST	SP	ST×SP	ST	SP	ST×SP
	NS	1.5	NS	19.1	NS	27.0	1.95	NS	2.75	6.37	6.37	9.01

FW = Fresh weight, ST = Storage temperature, SP = Storage period, ST×SP = Temperature × storage period interaction, NS = Not significant,

n = 4 ± SD, 10 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by lower case, superscript letters within the comparison between storage temperature and period. Significant difference between the mean storage temperatures with each period is represented by different upper case, superscript letters. All of the lettering is separate for pulp and peel and for different compounds.

Table 7.4. The concentrations of ascorbic acid and total carotenoids in the pulp of ripe mangoes exposed to different cold storage temperatures and durations

Storage temperature	Ascorbic acid (mg kg^{-1} FW)			Total carotenoids (mg kg^{-1} FW)		
	Storage period (d)			Storage period (d)		
	12	24	Mean (ST)	12	24	Mean (ST)
5 ℃	329.0 ± 17.11^{a}	378.0 ± 25.7^{b}	353.5^{A}	19.5±1.1	21.1±0.5	20.3^{B}
13 ℃	332.0 ± 12.6^{a}	358.0 ± 25.4^{b}	345.0^{A}	25.8±2.6	23.2±1.4	24.5^{A}
Mean (SP)	330.5^{B}	368.0^{A}		22.65^{A}	22.15^{A}	
LSD ($P \leq 0.05$)	ST	SP	ST×SP	ST	SP	ST×SP
	NS	17.35	24.54	2.03	NS	NS

FW = Fresh weight, ST = Storage temperature, SP = Storage period, ST×SP = Temperature × storage period interaction, NS = Not significant, n = 4 ± SD, 10 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by lower case, superscript letters within the comparison between storage temperature and period. Significant difference between the mean storage temperatures with each period is represented by different upper case, superscript letters. All of the lettering is separate for pulp and peel and for different compounds.

7.3.2.4 Ascorbic acid and total carotenoids

The concentration of ascorbic acid in the pulp of ripe fruit was not affected by the storage temperature ($P \leq 0.05$) (Table 7.4); however, the effect of cold storage duration and the interaction between storage temperature and duration were found to be significant ($P \leq 0.05$). Its concentration in the pulp was significantly higher at 24 d cold storage compared to 12 d, irrespective of the storage temperature (Table 7.4).

The concentration of total carotenoids in the pulp was significantly influenced only by the storage temperature ($P \leq 0.05$) (Table 7.4). When averaged over storage periods, the mean concentration of total carotenoids in the pulp of ripe fruit was significantly higher when stored at 13 °C than in the fruit previously stored at 5 °C.

7.4 Discussion

Browning of the skin, poor colour development, prominent lenticels and uneven ripening were the symptoms of chilling injury observed in the ripe fruit which were stored at 5 °C for 24 d. However, none of these symptoms appeared in ripe fruit subjected to any other treatment. Earlier, Nair et al. (2003) also reported similar symptoms and severity of chilling injury on 'Kensington Pride' mango fruit when stored at 5 °C for 20 d.

This study revealed that, the storage of mango fruit at 5 °C prior to ripening could significantly increase the concentration of lupeol in both pulp and peel at ripe stage (Table 7.1). On the other hand, storage at 13 °C prior to ripening could significantly increase the concentrations of mangiferin (Table 7.1), chlorogenic acid, ferulic acid and caffeic acid in the peel (Table 7.2) and gallic and vanillic acids in both pulp and peel of ripe mango fruit.

Recently, exposure of fruit and vegetables to low temperature stress during storage has been identified as a treatment for triggering the production of desirable dietary phytochemicals with significant health benefits (Schreiner and Huyskens-Keil, 2006). The biosynthetic pathways of terpenes and phenols are activated after an

elicitor treatment by inducing the activity of the enzyme, phenylalanine ammonia lyase (PAL) (Cisneros-Zevallos, 2003; Ruiz-García and Gómez-Plaza, 2013). Possibly, the enhanced activity of enzyme PAL may have contributed to the increase in the concentrations of lupeol, mangiferin and phenolic acids seen in the present study. Similarly, Rivera-Pastrana et al. (2010) reported that the concentrations of both ferulic and caffeic acids were better retained or even increased under low-temperature storage (5 °C) in 'Maradol' papaya fruit compared to those stored at 25 °C.

In our study, low temperature storage for 24 d at 5 °C or 13 °C apparently induces the production and/or retention of vanillic and caffeic acids compared to 12 d (Table 7.2). In contrast, storage for 24 d at chilling (5 °C) and non-chilling (13 °C) low temperatures gave lower retention of gallic and chlorogenic acids in pulp compared to 12 d (Table 7.2). Previously, Nair and Singh (2009) reported that rate of respiration in 'Kensington Pride' mango fruit was significantly reduced when stored at 5 °C when compared to 15 °C and 20 °C. It is known that the polyphenol biosynthesis is triggered during ripening to help shield the oxidative stress at respiratory climax (Masibo and He, 2008). Thus, the suppression of the rate of respiration during low temperature storage could possibly have led to the lower production of some phenolic acids in fruit stored at chilling temperatures (5 °C) and/ or for 24 d. Thus, the final concentration of phytochemicals could be a result of a balance between the activation of secondary metabolite biosynthesis as a defense mechanism and the respiratory demand to shield the oxidative stress during respiratory climax.

The current study showed that the effect of storage temperature was not significant on the concentration of total phenols in the pulp of ripe 'Kensington Pride' mango fruit (Table 7.3). Heredia and Cisneros-Zevallos (2009) also observed non-significant influence of elicitor treatments on the concentration of total phenols in some of the fruit and vegetables they investigated. They suggest that this apparently non responsive behaviour of some fresh produce to abiotic stress might be due to similar kinetics of phenolic synthesis and degradation which balances out the final concentration. On the other hand, there can be a preferential diversion of soluble

phenolics to insoluble forms such as lignin and suberin as a part of a defence response to help strengthen the cell walls of plant tissues (Reyes et al., 2007). This mechanism possibly has occurred in the present study. The changes in the concentration of total phenols after elicitor application are known to be tissue dependent (Reyes et al., 2007). Thus, a significantly higher mean concentration of total phenols was noted in the peel of ripe mango fruit previously stored at 13 °C compared to 5 °C (Table 7.3).

The mean total antioxidant capacity of the fruit pulp was significantly higher in fruit stored at 5 °C than at 13 °C (Table 7.3). The opposite effect of storage temperature on total antioxidant capacity was found in the peel (Table 3). This effect in the peel is most likely related to the higher concentration of total phenols at 13 °C (Table 7.3). Previously, Reyes et al. (2007) identified a similar trend of changes in the concentration of total phenols and the total antioxidant capacity in sixteen fruit and vegetables, finding a significantly positive correlation between the total phenol content and the antioxidant potential.

The concentration of ascorbic acid in the ripe pulp of 'Kensington Pride' mango fruit was not significantly influenced by the storage temperature (Table 7.4). However, it increased with extended storage time. Similarly, Thomas and Janave (1975) reported a net increase in the concentration of ascorbic acid in cold stored (7 °C) 'Alphonso' mangoes for 16 - 43 d compared to the fruit kept at tropical ambient temperature (28 - 32 °C).

A significantly lower level of total carotenoids in the pulp of ripe fruit was found at 5 °C, but no effect of storage duration was observed (Table 7.4). Similarly, a significant reduction in carotenoid formation was noted in ripe 'Alphonso' mango fruit stored at 7 °C for 16 - 43 d compared to 28 - 32 °C (Thomas and Janave, 1975). Vazquez-Salinas and Lakshminarayana (1985) also reported a significantly higher concentration of carotenoids in 'Keitt', 'Haden', 'Irwin' and 'Kent' mango fruit stored at 22 - 28 °C compared to those stored at 16 - 20 °C for 2 - 10 d. According to Saltveit (1999), the maximal carotenoid level during ripening period of climacteric fruit coincides with respiration and ethylene climaxes. As mentioned earlier, Nair

and Singh (2009) reported significantly lower rates of ethylene production and respiration in the 'Kensington Pride' mango fruit stored at 5 °C for 20 d prior to ripening. Therefore; the lower levels of total carotenoids at chilling storage temperature (5 °C) may have been associated with the reduced rates of respiration and ethylene production.

In general it has been reported that the peel of mango fruit has higher levels of dietary polyphenols and lupeol than pulp (Berardini et al., 2005a; Kim et al., 2010a; Masibo and He, 2008; Ruiz-Montañez et al., 2014; Srivastava et al., 2015). In agreement, higher concentrations of lupeol, mangiferin (Table 7.1), phenolic acids *viz*. gallic, cholorogenic, vanillic, ferulic and caffeic (Table 7.2), total phenols and the total antioxidant capacity (Table 7.3) were observed in this study in the peel of mango fruit than the pulp across all treatments.

7.5 Conclusion

In this study, a diverse range of effects of chill- and standard low storage temperatures and durations were observed in the concentrations of health-promoting compounds in mango pulp and peel. Chilling injury symptoms were only developed in the fruit stored at 5 °C for 24 d. The storage at 5 °C triggered the production of lupeol in both pulp and peel and chlorogenic acid and caffeic acid in the pulp. Whereas, the concentrations of mangiferin, gallic acid, chlorogenic acid, vanillic acid, ferulic acid, caffeic acid, total phenols, total antioxidants and total carotenoids in the peel were significantly higher after the storage at 13 °C compared to 5 °C. The concentration of ascorbic acid was not influenced by the storage temperature. The concentrations of lupeol and chlorogenic caid in both pulp and peel and the concentration of gallic acid in the pulp were significantly lower after storage for 24 d compared to 12 d, whereas, the concentrations of vanillic acid, total phenols, total antioxidants and ascorbic acid in the pulp and caffeic acid in both pulp and peel were significantly higher after storage for 24 d compared to 12 d. This is the first report on the effect of chilling and standard non chilling low temperature storage on the concentrations of lupeol, mangiferin and phenolic profile of any mango variety. Further knowledge in dynamics of the activity of enzyme phenylalanine ammonia-

lyase (PAL) during storage at above temperatures will provide a better insight to the mechanisms underlying the enhanced production of phytochemicals in mango fruit. Chill-temperature (5 °C) storage for 12 d prior to ripening seems to be a promising way to increase the concentration of lupeol in mango fruit. Peel of mango stored at either chill or standard low temperature may also provide a good source of health-promoting compounds for food processing and nutraceutical industries to cater for health-oriented markets.

CHAPTER 8

Postharvest application of methyl jasmonate, salicylic acid and nitric oxide regulates the concentration of health beneficial phytochemicals in ripe mango fruit

ABSTRACT

Postharvest elicitor treatments could be used to increase the levels of bioactive compounds in fruit. Green mature mango fruit were fumigated with methyl jasmonate (MeJA), nitric oxide (NO) or dipped in salicylic acid (SA) to investigate their effects on the concentrations of nutraceuticals in ripe fruit. The concentrations of lupeol and caffeic acid in the peel; mangiferin, gallic acid, chlorogenic acid, total phenols and total antioxidants in both pulp and peel and total carotenoids and ascorbic acid in the pulp were significantly ($P \leq 0.05$) higher with MeJA (10^{-5} and/or 10^{-4}M) fumigation compared to control. The concentration of lupeol in the pulp was significantly increased with 2 and 3 mmol L^{-1} SA dip whilst, mangiferin and ferulic acid in pulp and peel were significantly increased with 1 and 2 mmol L^{-1} SA dip compared to control. The concentration of vanillic acid in the pulp was significantly higher with all SA treatments compared to control. The concentrations of chlorogenic acid in both pulp and peel and total carotenoids and ascorbic acid in the pulp were increased with 2 mmol L^{-1} SA treatment. NO (20 and/or 40 μL L^{-1}) fumigation increased the concentrations of lupeol, mangiferin, gallic and chlorogenic acids in the pulp and peel, vanillic, ferulic and caffeic acids, total phenols, total antioxidants, total carotenoids and ascorbic acid in the pulp compared to the control. For the first time, MeJA, SA and NO were identified as effective elicitors to trigger the production of nutraceuticals in ripe mango fruit.

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KEYWORDS: *Mangifera indica* L.; elicitors; health-promoting compounds; MeJA; SA; NO

8.1 Introduction

Mango (*Mangifera indica* L.) fruit is a rich source of bioactive compounds such as lupeol, mangiferin, and phenolic acids that are well-known for their health benefits (Gallo and Sarachine, 2009; Masibo and He, 2009). Owing to the mounting awareness of these health benefits, consumers are more concerned about the levels of bioactive compounds in fruit currently than in the past (Schreiner and Huyskens-Keil, 2006). Therefore value addition by increasing concentrations of health-promoting compounds in fresh fruit and vegetables offers attractive opportunities for growers as well as food processors to cater for this expanding demand. Further, this new trend in demand has catalysed the development of novel technologies which ensure the production of fresh produce with higher concentrations of desired phytochemicals (Cisneros-Zevallos, 2003).

Varying from simple cultural practices such as pruning, fruit thinning or deficit irrigation to highly technical methods such as genetic engineering and plant cell culture of the specific cells which produce specific bioactive compounds have been developed to enhance the concentration of secondary metabolites in plants (Ruiz-García and Gómez-Plaza, 2013). Even though genetic engineering is a promising tool for the production of bioactive compounds, there is a significant disapproval from general public, environmentalists, and lobby groups over health and safety concerns of this technique in addition to potential ecological imbalance, high cost and lengthy procedures involved (Flinn and Zavon, 2004). Production of plant secondary metabolites by plant cell culture technology also face several biological and biotechnological limitations including low yields (Zhao et al., 2005). Since the major role of plant secondary metabolites is to protect plants from various biotic and abiotic stresses such as insect herbivores, pathogens, water deficit and extreme temperatures, the stress induction has been used to stimulate the production of these compounds (Zhao et al., 2005).

Many reports claim that plants subjected to abiotic stresses such as low temperature, heat, deficit irrigation could synthesize higher concentrations of secondary metabolites with potential use in health beneficial food production as well as raw materials and nutraceuticals (Jacobo-Velázquez and Cisneros-Zevallos, 2012; Schriener and Huyskens-Keil, 2006). Similarly, targeted postharvest elicitor treatments such as methyl jasmonate (MeJA), salicylic acid (SA) and ethylene could be used to produce fruit and vegetables with higher concentrations of phytochemicals to consume either fresh or to be used as raw materials in functional food industry (Schreiner and Huyskens-Keil, 2006). Nitric oxide (NO) has been recently recognized as a signal compound in plants, which can also be used as a postharvest elicitor (Zhao et al., 2005).The use of abiotic stresses such as chemical elicitors in the production of secondary metabolites is considered as a practical, effective and safe tool when compared with other available biotechnological strategies such as genetic engineering (Cisneros-Zevallos, 2003; Ruiz-García, Y and Gómez-Plaza, 2013).

Low temperature, heat treatment, ultraviolet and gamma irradiation and altered gas composition are categorized as physical elicitors, while plant signalling molecules such as MeJA, SA and ethylene are considered as the dominating chemical elicitors (Schreiner and Huyskens-Keil, 2006). The signal transduction network that occurs after an elicitor treatment induces the biosynthetic pathways of important secondary metabolites (Cisneros-Zevallos, 2003). Jasmonic acid (JA) signalling pathway induces the accumulation of a wide range of plant secondary products including terpenoids, flavonoids, alkaloids and phenylpropanoids as a part of the plant defence responses (Zhao et al., 2005; Rohwer and Erwin, 2008; van der Fits and Memelink, 2000). SA (Halim et al., 2006) and NO (Zhao et al., 2005) are also crucial signalling molecules required for the expression of plant defence responses. Biosynthetic genes responsible for plant secondary metabolism are expressed after the receptor binding of these signals (Zhao et al., 2005). Biosynthesis of polyphenols is initiated by the deamination of phenylalanine, which is catalysed by phenylalanine ammonia lyase (PAL) (Ruiz-García and Gómez-Plaza, 2013). The activity of PAL is induced by postharvest elicitors or abiotic stresses, causing an increase in the concentration of phenolic compounds in plant tissues (Cisneros-Zevallos, 2003).

The effects of MeJA on chilling injury, rate of respiration, fruit colour, firmness, soluble solids concentration, sugars and acidity in cold stored fruit of mango cultivars 'Tommy Atkins' (Gonzalez-Aguilar et al., 2000); 'Kent' (Gonzalez-Aguilar et al., 2001) and 'Kensington Pride' (Lalel et al., 2003b) have previously been reported. Earlier, the effect of MeJA on the concentrations of fatty acids and aroma volatile compounds and ethylene production in 'Kensington Pride' mango fruit has also been reported (Lalel et al., 2003b). However, the influence of MeJA as a postharvest elicitor in inducing the production of health-promoting compounds such as lupeol, mangiferin and phenolic acids in mango fruit stored at room temperature is yet unknown.

The effect of SA on disease resistance, skin colour and firmness of 'Kensington Pride' mango fruit was reported by Joyce et al. (2001). Disease resistance, activities of peroxidase (POD), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) and the concentration of total phenolic compounds in 'Matisu' mango fruit with SA application has been reported by Zeng et al. (2006). Similarly, the effect of SA on chilling injury, rate of respiration and ethylene production, total carotenoids, total phenolics, total antioxidant activity and decay incidence in cold- stored 'Chausa' mango fruit has also been reported (Barman and Asrey, 2014). No research work has however been reported on the effects of SA application as a postharvest elicitor on the production of lupeol, mangiferin and phenolic acids in mango fruit stored at room temperature.

The effect of NO on chilling injury, rate of respiration, rate of ethylene production, colour, firmness, sugars, ascorbic acid, total carotenoids and total antioxidant capacity in 'Kensington Pride' mango fruit kept in cold storage have been previously reported (Zaharah and Singh, 2011b). However, the effects of exogenously applied NO on the production of lupeol, mangiferin and phenolic acids in mango fruit under ambient conditions warrant to be investigated.

In this study, it was hypothesised that the postharvest application of chemical elicitors such as MeJA, SA and NO will enhance the levels of desired bioactive compounds in mango fruit. Therefore, the effects of postharvest application of

MeJA, SA and NO on modulating the concentrations of lupeol, mangiferin, phenolic acids, total phenols, total antioxidant capacity, total carotenoids, and ascorbic acid in ripe mango fruit was investigated.

8.2 Materials and methods

Deatiled methodologies are given in Chapter 3 (page 39).

Three independent experiments were carried out to investigate the effects of postharvest application of different concentrations of MeJA, SA and NO on the levels of health-promoting compounds in ripe mango fruit.

8.2.1 Materials

Fruit

Hard green mature 'Kensington Pride' mango fruit (light cream pulp, 100% green peel, firmness: 167±1 N) were harvested from a commercial orchard in Chittering (31° 45´S, 116° 1´E), Western Australia and transported within 2 h to the laboratory at Perth on the 10th of February 2016. Mangoes free from defects, physical injuries and visible disease symptoms were used in different experiments.

Experimental design and compounds analyzed

Each experiment was designed following a completely randomized design (CRD). All the treatments were replicated three times and ten fruit were included in each replication. At eating soft stage, the concentrations of lupeol, mangiferin, phenolic acids (gallic, chlorogenic, vanillic, ferulic and caffeic), total phenols and total antioxidants in both pulp and peel and total carotenoids and ascorbic acid in pulp were estimated.

Experiment 1: Effects of exogenous application of different concentrations of MeJA on the levels of health-promoting compounds

The fruit were fumigated with different concentrations of MeJA (10^{-4} M, 10^{-5} M and 10^{-6} M) based on the head space in sealed plastic containers of 60.0 L total volume for 24 h at ambient temperature (21 ± 1 °C). The required amount of MeJA was loaded as a spot on a Whatman no. 1 filter paper (Gonzalez-Aguilar et al., 2000). Soda lime (25.0 g) in a petri dish was kept in each container to absorb excessive CO_2. A mini portable fan was operated inside the plastic container prior to sealing to ensure equal distribution of the MeJA vapour. Control fruit were sealed in a similar plastic container (60.0 L) for the same duration under the same conditions without any MeJA. After 24 h, the fruit were removed from the containers, packed in to cardboard boxes and then kept in a well ventilated room at ambient temperature (21 ± 1 °C) until eating soft stage.

Experiment 2: Effects of different concentrations of salicylic acid (SA) on the levels of health-promoting compounds

The fruit were dipped in aqueous solutions containing different concentrations of SA (1.0 mmol L^{-1}, 2.0 mmol L^{-1} and 3.0 mmol L^{-1}) with Tween® 20 as a surfactant (50.0 μL L^{-1}) in plastic tubs for 10 min at ambient temperature (21 ± 1 °C) as described by Joyce et al. (2001). Control fruit were dipped in the same volume of distilled water for 10 min under the same conditions. The fruit were air dried and then allowed to ripen until eating soft stage at ambient temperature (21 ± 1 °C).

Experiment 3: Effects of different concentrations of nitric oxide (NO) fumigation on the levels of health-promoting compounds

Fruit were fumigated with different concentrations of NO (10.0, 20.0 and 40.0 μL L^{-1}) in sealed plastic containers (60.0 L) for 2 h at ambient temperature (21 ± 1 °C). The required volume of NO gas to fill the head space was injected through an injection port fitted on the lid of the container as described previously by Zaharah and Singh (2011). A petri dish containing soda lime (25.0 g) was placed into

each container to absorb excessive CO_2. A mini portable fan was operated inside before sealing each of the containers for equal distribution of NO. Control fruit were sealed in a similar plastic container (60.0 L) for the same duration under the same conditions without NO gas. Following fumigation, the fruit were removed from the container and then allowed to ripen at ambient temperature (21 ± 1 °C) until eating soft stage.

Sample preparation

At eating soft stage, representative samples of pulp and peel of 10 fruit in each replication prepared as described in Chapter 3, Section 3.2 were immediately stored at -80 °C. The concentrations of total phenols, total antioxidants, ascorbic acid and total carotenoids were determined using thawed samples of each replication. Freeze dried and powdered samples were used for the determination of the concentrations of lupeol, mangiferin and phenolic acids.

8.2.2 Chemicals

All the reagents, MeJA, SA and the standards of respective bioactive compounds were purchased from Sigma Aldrich (St. Louis, MO, USA) except acetonitrile, n-hexane and methanol (Thermo Fisher Scientific, Taren Point, NSW, Australia). Chemicals used were of HPLC grade. Nitric oxide in nitrogen was purchased from BOC Australia, Sydney, NSW, Australia.

8.2.3 Determination of health-promoting compounds

8.2.3.1 Lupeol

The methods described previously by Ruiz-Montañez et al. (2014) and Oliveira et al. (2012) were followed for the extraction and quantification of lupeol from freeze-dried mango pulp/peel samples with slight modifications as described in Chapter 3, Section 3.5.1. The concentration of lupeol was calculated using a standard curve and expressed as mg kg^{-1} dry weight basis.

8.2.3.2 Mangiferin and phenolic acids

The method reported earlier by Palafox-Carlos et al. (2012a) was followed for the determination of the levels of mangiferin and phenolic acids in freeze-dried pulp and peel samples of mango fruit with some modifications as detailed in Chapter 3, Section 3.5.2. The concentrations of individual polyphenols were quantified using respective standard curves and expressed as mg kg^{-1} dry weight basis.

8.2.3.3 Total phenols

Folin - Ciocalteu method reported previously by Robles-Sànchez et al. (2009) was used to estimate the total phenol concentration. The detailed procedure has been described in Chapter 3, Section 3.5.3. The total phenol concentration was quantified using a gallic acid standard curve and expressed in g gallic acid equivalents (GAE) kg^{-1} fresh weight basis.

8.2.3.4. Total antioxidant capacity

The total antioxidant capacity of respective pulp and peel samples was estimated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay detailed in Chapter 3, Section 3.5.4. Total antioxidant capacity in the pulp and peel were quantified using a standard curve of Trolox (6-hydroxy-2, 5, 7, 8-tetramethychromane-2-carboxylic acid) and expressed as mmol trolox equivalent antioxidant capacity (TEAC) kg^{-1} fresh weight basis.

8.2.3.5 Total carotenoids

The total carotenoid concentration of ripe mango pulp was determined by the method described earlier by Lalel et al. (2003a) under reduced light conditions using precautions to prevent photo-degradation of carotenoids as described in Chapter 3, Section 3.5.6. The total carotenoid concentration was estimated using a β-carotene standard curve and expressed as mg kg^{-1} on fresh weight basis.

8.2.3.6 Ascorbic acid

The method detailed in Chapter 3, Section 3.5.6 was followed to determine the concentration of ascorbic acid in pulp samples. The quantification was carried out using a standard curve of L-ascorbic acid and the concentration of ascorbic acid was expressed as mg kg^{-1} fresh weight basis.

8.2.4 Statistical analysis

The data of each experiment were subjected to one-way ANOVA using GenStat version 14.0 (Lawes Agricultural Trust, Rothamsted, UK) software. Duncan multiple comparison test was used to calculate the significant differences among treatments. The results were expressed as means ± SD.

8.3 Results

8.3.1 Effects of methyl jasmonate (MeJA) on the concentrations of health-promoting compounds

8.3.1.1 Lupeol and mangiferin

A significantly higher concentration of lupeol was recorded in the peel of fruit fumigated with MeJA (10^{-5} M and 10^{-4} M) compared to the untreated control and 10^{-6} M MeJA treatment (Table. 8.1). On the other hand, a significantly lower concentration of this compound was observed in the pulp of fruit fumigated with 10^{-5} M MeJA compared to the untreated control and other MeJA concentrations (Table. 8.1). The highest concentrations of mangiferin in pulp were recorded in the fruit fumigated with 10^{-5} M and 10^{-4} M MeJA compared to untreated control and 10^{-6} M MeJA treatment, whilst the highest concentration was recorded in the peel of fruit fumigated with 10^{-5} M MeJA (Table 8.1).

Table 8.1. Effects of methyl jasmonate (MeJA) fumigation on the concentrations of lupeol, mangiferin,gallic, chlorogenic, vanillic, ferulic and caffeic acids in the pulp and peel of ripe fruit

Compound	Pulp				Peel			
(mg kg^{-1} DW)	Control	10^{-6} M	10^{-5} M	10^{-4} M	Control	10^{-6} M	10^{-5} M	10^{-4} M
Lupeol	9.5±0.6^{b}	9.1±0.3^{b}	6.7±0.2^{a}	8.7±0.7^{b}	10.1±1.3^{a}	15.8±1.6^{ab}	21.6±4.1^{b}	20.2±0.9^{b}
Mangiferin	9.9±0.6^{a}	10.7±0.2^{a}	11.9±0.3^{b}	11.6±0.2^{b}	27.1±4.0^{a}	30.6±2.5^{ab}	37.5±0.6^{c}	33.6±1.9^{bc}
Gallic	120.4±7.5^{a}	215.0±19.6^{b}	310.4±32.0^{c}	282.7±23.1^{c}	691.9±36.6^{a}	823.7±31.4^{b}	1033.5±34.7^{c}	1015.3±26.1^{c}
Chlorogenic	179.0±6.7^{a}	151.1±4.2^{a}	296.0±21.8^{c}	223.0±13.6^{b}	1004.0±96.9^{a}	1122.0±61.8^{a}	1308.0±50.4^{b}	1429.0±63.4^{b}
Vanillic	65.6±10.4	74.8±3.5	79.7±4.4	76.7±8.2	168.6±5.2	136.9±33.8	204.3±29.4	188.9±26.6
Ferulic	62.9±18.5	55.0±17.1	72.0±18.0	79.1±10.0	296.7±60.6	317.7±51.4	492.3±77.9	525.7±44.0
Caffeic	0.6±0.2	0.7±0.1	1.1±0.2	1.0±0.1	5.7±1.3^{a}	11.9±2.4^{a}	30.0±5.4^{b}	26.2±2.4^{b}

DW = Dry weight, n = 3 ± SD, 10 fruit per replication. Means followed by different lower-case superscript letters along rows for pulp and peel separately are significantly different by Duncan multiple comparison test at $P \leq 0.05$. Lettering for pulp and peel is independent of each other. No lettering along a row denotes non significance.

Table 8.2. Effects of methyl jasmonate fumigation on the concentrations of total phenols and total antioxidants, in the pulp and peel as well as total carotenoids and ascorbic acid in the pulp of ripe fruit

Compound (FW)	Pulp				Peel			
	Control	10^{-6} M	10^{-5} M	10^{-4} M	Control	10^{-6} M	10^{-5} M	10^{-4} M
Total phenols (g GAE kg^{-1})	4.5±0.2^{a}	3.9±0.4^{a}	6.6±0.8^{b}	5.6±0.2^{b}	158.1±18.2^{a}	177.6±15.3^{ab}	213.0±17.0^{b}	256.7±14.4^{c}
Total antioxidants (mmol TEAC kg^{-1})	21.5±2.3^{a}	20.1±0.2^{a}	19.9±0.2^{a}	25.1±0.2^{b}	199.0±2.4^{a}	200.7±2.4^{a}	251.3±2.4^{b}	215.4±23.0^{a}
Total carotenoids (mg kg^{-1})	13.9±2.2^{a}	27.0±0.8^{c}	20.4±0.2^{b}	25.4±1.1^{c}	NE	NE	NE	NE
Ascorbic acid (mg kg^{-1})	267.0±8.1^{a}	289.0±23.2^{ab}	336.0±51.1^{b}	323.0±17.1^{b}	NE	NE	NE	NE

FW = Fresh weight, n = 3 ± SD, 10 fruit per replication. Means followed by different lower-case superscript letters along rows for pulp and peel separately are significantly different by Duncan multiple comparison test at $P \leq 0.05$. Lettering for pulp and peel is independent of each other. No lettering along a row denotes non significance. NE = Not estimated.

8.3.1.2 Phenolic acids

The concentrations of gallic and chlorogenic acids in both pulp and peel and caffeic acid in the peel of fruit fumigated with 10^{-5} M and 10^{-4} M MeJA were significantly higher ($P \leq 0.05$) than that of untreated control and 10^{-6} M MeJA treatment (Table 8.1). No effect of MeJA was observed on the concentrations of vanillic and ferulic acids in either pulp or peel and caffeic acid in the pulp of ripe fruit compared to the untreated control (Table 8.1).

8.3.1.3 Total phenols, total antioxidant capacity, total carotenoids and ascorbic acid

MeJA (10^{-5} M and 10^{-4} M) fumigation treatments resulted in higher concentrations of total phenols in the pulp and the peel compared to the control and 10^{-6} M MeJA treatment (Table 8.2). The total antioxidant capacity in the pulp and peel of the fruit was the highest when fumigated with 10^{-4} M MeJA compared to the control and all other treatments (Table 8.2). All the MeJA fumigation treatments significantly increased the concentration of total carotenoids in the pulp of the ripe mango fruit compared to the control (Table 8.2). The concentrations of ascorbic acid were significantly higher in the pulp of ripe fruit fumigated with 10^{-5} M and 10^{-4} M MeJA compared to the untreated control and 10^{-6} M MeJA treatment (Table 8.2).

8.3.2 Effects of salicylic acid (SA) on the concentrations of health-promoting compounds

8.3.2.1 Lupeol and mangiferin

The highest concentrations of lupeol in the pulp were recorded in fruit treated with 2 mmol L^{-1} and 3 mmol L^{-1} SA compared to the control and 1 mmol L^{-1} SA treatment (Table 8.3). However, the lupeol level was significantly lower in the peel of the fruit treated with SA compared to the control. The highest concentration of mangiferin in the pulp was observed in the fruit treated with 1 mmol L^{-1} and 2 mmol L^{-1} SA, whereas it was highest in the peel of the fruit treated with 1 mmol L^{-1} SA (Table 8.3).

8.3.2.2 Phenolic acids

The fruit treated with 2 mmol L^{-1} SA exhibited the highest levels of chlorogenic acid in the pulp and peel compared to the control and all other SA treatments (Table 8.3). A significantly higher concentration of vanillic acid was recorded in the pulp of the fruit treated with SA (1 mmol $L^{-1,}$ 2 mmol L^{-1} and 3 mmol L^{-1}) compared to the untreated control. Whilst, SA treatment did not influence the levels of vanillic acid in the peel compared to the untreated control. The highest concentrations of ferulic acid in both pulp and peel were recorded in the fruit treated with 1 mmol L^{-1} and 2 mmol L^{-1} SA compared to the control and 3 mmol L^{-1} SA treatment (Table 8.3). In both pulp and peel SA treatment resulted in significantly lower concentrations of gallic acid (Table 8.3).

8.3.2.3 Total phenols, total antioxidant capacity, total carotenoids and ascorbic acid

The concentrations of total phenols and total antioxidant capacity in the pulp of ripe mango fruit were not significantly affected with any of the SA dip treatments compared to the control (Table 8.4). All the SA treatments significantly ($P \leq 0.05$) reduced the concentration of the total phenols in the peel of the ripe fruit compared to the untreated control (Table 8.4), whereas SA treatments did not show any positive effects on the total antioxidant capacities in the peel of ripe mango fruit compared to the control (Table 8.4). The concentration of total carotenoids in the pulp of ripe fruit was significantly ($P \leq 0.05$) reduced with all the SA treatments compared to the untreated control (Table 8.4). The concentration of ascorbic acid in the pulp of ripe fruit was highest when treated with 1 mmol L^{-1} SA compared to the untreated control and all other treatments (Table 8.4).

Table 8.3. Effects of salicylic acid dip treatment on the concentrations of lupeol, mangiferin, gallic, chlorogenic, vanillic, ferulic and caffeic in the pulp and peel of ripe fruit

Compound	Pulp				Peel			
(mg kg^{-1} *)	Control	1 mM L^{-1}	2 mM L^{-1}	3 mM L^{-1}	Control	1 mM L^{-1}	2 mM L^{-1}	3 mM L^{-1}
Lupeol	8.2±0.8^{a}	8.7±0.3^{a}	21.6±4.1^{b}	15.8±1.6^{b}	32.2±2.9^{b}	25.8±1.8^{a}	24.6±3.7^{a}	22.9±2.1^{a}
Mangiferin	10.3±0.1^{a}	11.1±0.1^{b}	11.3±0.2^{b}	10.0±0.3^{a}	22.2±0.4^{ab}	26.4±3.7^{b}	23.3±1.5^{ab}	21.4±0.7^{a}
Gallic	283.3±58.2^{b}	220.4±19.3^{ab}	237.0±18.7^{ab}	174.0±19.8^{a}	1049.3±31.5^{b}	859.0±37.2^{a}	904.1±35.4^{a}	887.0±19.4^{a}
Chlorogenic	441.7±61.2^{b}	556.0±60.6^{bc}	639.7±26.6^{c}	240.0±71.2^{a}	1662.0±36.5^{a}	1624.0±149.9^{a}	1971.0±93.5^{b}	1648.0±115.4^{a}
Vanillic	64.3±5.0^{a}	128.9±19.0^{b}	136.0±11.5^{b}	116.2±8.7^{b}	207.5±26.5	188.6±19.1	184.4±20.8	190.5±20.0
Ferulic	27.0±9.0^{a}	105.4±3.5^{b}	91.9±13.1^{b}	24.8±14.4^{a}	144.6±20.5^{a}	446.5±11.2^{b}	448.8±28.0^{b}	91.5±36.4^{a}
Caffeic	1.71±0.8	2.07±0.4	2.41±0.5	1.19±0.05	29.9±2.9	29.9±2.1	29.9±1.0	25.6±1.3

* Dry weight, n = 3 ± SD, 10 fruit per replication. Means followed by different lower-case superscript letters along rows for pulp and peel separately are significantly different by Duncan multiple comparison test at $P \leq 0.05$. Lettering for pulp and peel is independent of each other.

Table 8.4. Effects of salicylic acid SA on the concentrations of total phenols, total antioxidants, total carotenoids and ascorbic acid

Compound (FW)	Pulp				Peel			
	Control	1 mM L^{-1}	2 mM L^{-1}	3 mM L^{-1}	Control	1 mM L^{-1}	2 mM L^{-1}	3 mM L^{-1}
Total phenols (g GAE kg^{-1})	5.6±0.7	5.2±1.0	5.3±0.3	6.1±0.5	248.0±14.3^{b}	130.8±25.0^{a}	113.3±8.3^{a}	151.3±10.3^{a}
Total antioxidants (mmol TEAC kg^{-1})	26.5±0.3	24.5±0.7	26.7±2.6	26.4±0.2	358.6±4.1^{b}	251.3±9.5^{a}	241.2±23.9^{a}	358.1±2.4^{b}
Total carotenoids (mg kg^{-1})	47.6±4.0^{c}	26.2±3.7^{a}	26.9±1.4^{a}	36.9±2.4^{b}	NE	NE	NE	NE
Ascorbic acid (mg kg^{-1})	359.0±17.1^{b}	413.0±22.2^{c}	296.0±14.9^{a}	356.0±15.9^{b}	NE	NE	NE	NE

FW = Fresh weight, n = 3 ± SD, 10 fruit per replication. Means followed by different lower-case superscript letters along rows for pulp and peel separately are significantly different by Duncan multiple comparison test at $P \leq 0.05$. Lettering for pulp and peel is independent of each other. NE = Not estimated. No lettering along a row denotes non-significance.

8.3.3 Effects of nitric oxide (NO) on the concentrations of health-promoting compounds

8.3.3.1 Lupeol and mangiferin

The highest concentrations of lupeol and mangiferin were recorded in the pulp of the fruit fumigated with 20.0 μL L^{-1} NO compared to the untreated control and all other NO treatments (Table 8.5). Whilst, the fruit fumigated with 20.0 μL L^{-1} NO and 40.0 μL L^{-1} NO exhibited the highest concentrations of lupeol and mangiferin in the peel of ripe fruit compared to the control and 10.0 μL L^{-1} NO treatment (Table 8.5).

8.3.3.2 Phenolic acids

The highest concentrations of gallic, chlorogenic and caffeic acids were noted in the pulp of the ripe fruit fumigated with 40.0 μL L^{-1} NO compared to the control and all other NO treatments (Table 8.5). Whlist, the highest concentrations of vanillic and ferulic acids in the pulp of ripe fruit were recorded when the fruit were fumigated with 20.0 μL L^{-1} and 40.0 μL L^{-1} NO compared to the untreated control and 10.0 μL L^{-1} NO (Table 8.5). The concentration of gallic acid in the peel of ripe fruit was significantly highest when treated with 20.0 μL L^{-1} NO as compared to control and all other treatments. NO fumigation treatments (20.0 and 40.0 μL L^{-1}) resulted in significantly higher concentrations of chlorogenic acid in the peel compared to all the other treatments. Any of the NO treatments did not significantly affect the concentrations of vanillic, ferulic and caffeic acids in the peel of ripe fruit compared to the control (Table 8.5).

8.3.3.3 Total phenols, total antioxidant capacity, total carotenoids and ascorbic acid

The highest concentration of total phenols was recorded in the in the pulp of fruit fumigated with 40.0 μL L^{-1} compared to control and all other NO treatments (Table 8.6). NO fumigation did not resulted in any positive effects on the total antioxidant capacity in the peel and pulp and the concentration of total phenols in the peel of the

ripe fruit compared to the control (Table 8.6). The highest concentration of total carotenoids and ascorbic acid in the pulp of ripe fruit were recorded in the fruit fumigated with 10.0 μL L^{-1} NO and40.0 μL L^{-1} NO respectively compared to untreated control and all the other treatments (Table 8.6).

Table 8.5. Effects of nitric oxide (NO) fumigation on the concentrations of lupeol, mangiferin, gallic, chlorogenic, vanillic, ferulic and caffeic in the ripe fruit

Compound (DW)	Pulp (mg kg^{-1})				Peel (mg kg^{-1})			
	Control	10 μL L^{-1}	20 μL L^{-1}	40 μL L^{-1}	Control	10 μL L^{-1}	20 μL L^{-1}	40 μL L^{-1}
Lupeol	9.0±0.6^{a}	9.8±0.6^{a}	12.4±0.2^{c}	10.5±0.6^{b}	12.2±1.8^{a}	15.6±1.3^{a}	23.0±1.2^{b}	20.6±0.8^{b}
Mangiferin	10.2±0.1^{a}	11.2±0.1^{b}	12.6±0.2^{c}	11.4±0.1^{b}	26.3±3.3^{a}	32.6±1.0^{b}	38.4±1.1^{c}	37.8±0.7^{c}
Gallic	184.3±14.7^{a}	222.5±21.1^{b}	242.2±14.8^{b}	297.5±31.6^{c}	873.5±21.7^{a}	886.4±37.3^{a}	978.8±19.4^{b}	892.2±36.5^{a}
Chlorogenic	293.3±58.1^{a}	309.5±38.8^{a}	315.8±33.6^{a}	453.8±45.8^{b}	1379.0±89.9^{a}	1393.1±24.3^{a}	1597.3±51.2^{b}	1621.4±149.0^{b}
Vanillic	66.3±5.5^{a}	76.8±5.6^{a}	112.8±14.4^{b}	118.6±12.8^{b}	179.3±13.7	201.5±14.7	182.3±13.2	193.4±14.9
Ferulic	44.5±5.7^{a}	52.9±2.8^{a}	88.3±11.8^{b}	95.6±10.5^{b}	283.9±21.1	288.3±17.8	293.3±13.5	296.5±22.8
Caffeic	0.9±0.3^{a}	1.1±0.1^{a}	0.8±0.2^{a}	2.1±0.3^{b}	25.6±1.5	26.8±0.9	27.6±0.5	28.2±1.3

DW = Dry weight, n = 3 ± SD, 10 fruit per replication. Means followed by different lower-case superscript letters along rows for pulp and peel separately are significantly different by Duncan multiple comparison test at $P \leq 0.05$. Lettering for pulp and peel is independent of each other. No lettering along a row denotes non-significance.

Table 8.6. Effects of nitric oxide (NO) on the concentrations of total phenols, total antioxidants, total carotenoids and ascorbic acid

Compound (FW)	Pulp				Peel			
	Control	10 µL L^{-1}	20 µL L^{-1}	40 µL L^{-1}	Control	10 µL L^{-1}	20 µL L^{-1}	40 µL L^{-1}
Total phenols (g GAE kg^{-1})	4.5 ± 0.3^{a}	6.6 ± 1.2^{b}	6.2 ± 0.2^{ab}	12.2 ± 0.7^{c}	176.6 ± 5.0^{b}	136.2 ± 9.2^{a}	166.2 ± 7.3^{b}	130.8 ± 19.3^{a}
Total antioxidants (mmol TEAC kg^{-1})	28.6 ± 0.5^{ab}	25.2 ± 2.4^{a}	24.1 ± 2.5^{a}	29.9 ± 0.3^{b}	258.1±4.1	227.4±21.8	217.6±22.5	251.2±2.3
Total carotenoids (mg kg^{-1})	29.3 ± 0.8^{b}	44.7 ± 2.3^{d}	21.2 ± 0.9^{a}	37.8 ± 2.9^{c}	NE	NE	NE	NE
Ascorbic acid (mg kg^{-1})	299.0 ± 15.4^{a}	343.0 ± 32.9^{ab}	289.9 ± 12.5^{a}	365.0 ± 21.2^{b}	NE	NE	NE	NE

FW = Fresh weight, n = 3 ± SD, 10 fruit per replication. Means followed by different lower-case superscript letters along rows for pulp and peel separately are significantly different by Duncan multiple comparison test at $P \leq 0.05$. Lettering for pulp and peel is independent of each other. NE = Not estimated.

8.4 Discussion

The concentrations of lupeol were significantly higher in the peel of the ripe fruit fumigated with 10^{-5} and 10^{-4} M MeJA, whilst levels of lupeol were significantly higher in the pulp of the fruit treated with 2.0 and 3.0 mmol L^{-1} SA. NO (20.0 and 40.0 µL L^{-1}) fumigation resulted in the highest concentrations of lupeol in both pulp and peel of ripe fruit (Table 8.1, 8.3 and 8.5). The elevated levels of lupeol in both pulp and peel in the ripe fruit with these chemical elicitor treatments may be a result of the enhanced biosynthesis of terpenoids (Zhao et al., 2005; Rohwer and Erwin, 2008; van der Fits and Memelink, 2000). The higher concentrations of mangiferin recorded in both pulp and peel of ripe fruit fumigated with 10^{-5} M and 10^{-4} M MeJA and all NO treatments and the pulp of ripe mango fruit treated with 1.0 and 2.0 mmol L^{-1} SA (Table 8.1, 8.3 and 8.5) might be a result of these elicitor treatments providing the signals to initiate the flavanoid biosynthetic pathway (Zhao et al., 2005; Rohwer and Erwin, 2008; van der Fits and Memelink, 2000).

The significantly higher concentrations of different phenolic acids in the ripe mango fruit subjected to chemical elicitor treatments (MeJA, SA, NO) in the present study might be a result of the increased activity of phenylalanine ammonia lyase (PAL) which initiates the biosynthesis of phenolic acids (Rohwer and Erwin, 2008; van der Fits and Memelink, 2000; Zeng et al., 2006; Zhao et al., 2005). However, a significantly lower concentration of gallic acid was observed in both the pulp and peel of ripe mango fruit dipped in SA solutions compared to control (Table 8.3), while ferulic, caffeic and vanillic acids in peel and /or pulp were not affected by some elicitor treatments (Table 8.1, 8.3 and 8.5). Similar observations have earlier been reported, when pre-harvest application of MeJA could significantly increase the concentrations of ferulic acid and chlorogenic acid in cold stored plum fruit, whilst caffeic acid was not affected (Karaman et al., 2013), whereas in 'Cabernet Sauvignon' grape berries, the concentrations of gallic, chlorogenic, ferulic and caffeic acids were significantly increased with SA treatment (Chen et al., 2006). It appears from the experimental data that the influence of chemical elicitors on the concentrations of phenolic acids is both compound and pulp and peel tissue dependent.

In the present study, MeJA fumigation significantly increased the concentration of total phenols in both pulp and peel, whilst NO was effective in elevating levels of total phenols in

the pulp of ripe mango fruit (Table 8.2 and 8.6). However, the concentration of total phenols was significantly lower in the peel of ripe mango fruit when exposed to different concentrations of SA and NO (Table 8.4 and 8.6). In agreement with this observation, a reduction in the total phenol concentration had been reported in 'Matisu' mangoes when exposed to SA (Zeng et al., 2006). The biosynthesis of phenolic antioxidants is induced by the respiratory climax to balance out the oxidative stress (Masibo and He, 2008). Thus, the suppression of the respiratory climax by the application of SA (Srivastava and Dwivedi, 2000) together with the significant reduction in the concentration of gallic acid and probable diversion of soluble phenolics to insoluble phenolics to strengthen the cell wall as a defence mechanism (Reyes et al., 2007) could be the reasons for this reduction. Following the same trend as total phenolics, the total antioxidant capacity in the ripe peel was also significantly reduced when exposed to 1.0 and 2.0 mmol L^{-1} SA. Polyphenols are among the key contributors of the total antioxidant capacity of mangoes (Ma et al., 2011). Hence, the reduction in the concentration of total phenols with the postharvest application of SA may be claimed for this lower antioxidant capacity (Table 8.4). Gallic acid has been identified as the phenolic acid which has the highest contribution to the antioxidant capacity of mango fruit (Palafox-Carlos et al., 2012a). Thus, the lower concentration of gallic acid recorded after SA treatment could also have contributed to this reduction.

In the present study, the concentration of total carotenoids in the pulp was significantly higher after the fumigation with MeJA and NO, whilst the concentration of ascorbic acid was significantly increased by all three elicitor treatments (Table 8.2, 8.4 and 8.6). Previously, González–Aguilar et al. (2006) reported that MeJA promotes *β*-carotene synthesis. Further, Wolucka et al. (2005) reported that MeJA could enhance the transcription of genes in the *de novo* biosynthesis of ascorbic acid. NO may also induce the biosynthetic pathways of *β*-carotene and ascorbic acid in the same manner. In contrast, 1.0 and 2.0 mmol L^{-1} SA treatment significantly reduced the concentration of total carotenoids in the ripe pulp (Table 8.4). In a previous study, the colour change of 'Kensington Pride' mango fruit peel from green to yellow was significantly reduced by SA dip (Joyce et al., 2001). Both these observations suggest a reduction in the biosynthesis of carotenoids with SA treatment.

8.5 Conclusion

This study for the first time demonstrated the effects of postharvest MeJA, SA and NO treatments on the concentrations of health-promoting compounds in the pulp and peel of ripe mango fruit. MeJA fumigation (10^{-5} M and/or 10^{-4} M) significantly increased the concentrations of lupeol, mangiferin, gallic, chlorogenic, caffeic acids, total phenols, total antioxidants, total carotenoids and ascorbic acid in the pulp and/or peel of ripe mango fruit. Dip-treatment of SA (2 mmol L^{-1}) significantly increased the concentrations of lupeol, mangiferin, chlorogenic and ferulic acids in pulp and/or peel. The concentrations of lupeol, mangiferin, all phenolic acids, total phenols, total antioxidants, and ascorbic acid were increased with NO fumigation (20.0 and /or 40.0 μL L^{-1}). Thus, 10^{-5} M and/or 10^{-4} M MeJA, 2 mmol L^{-1} SA and 20.0 and/or 40.0 μL L^{-1} NO seem to be the best concentrations of above elicitors in promoting levels of different health promoting compound in the ripe mango fruit. In general, the postharvest application of chemical elicitors MeJA, SA and NO at suggested concentrations could be a practical, efficient and safe tool to increase the levels of health-beneficial phytochemicals in ripe mango fruit to cater for the growing consumer demand. Determination of the effect of these compounds on the activity of PAL and other key enzymes needed in the biosynthetic pathways of important phytochemicals in pulp and peel of mango fruit would help better manipulation of the concentration of health-promoting compound in future studies.

CHAPTER 9

General discussion, conclusions, recommendations and future prospects

9.1 Introduction

There is a growing interest on health-promoting compounds in fruit and vegetables owing to the increasing evidence of their ability in reducing the risk of chronic degenerative diseases (Scalbert et al., 2005; Schreiner and Huyskens-Keil, 2006). Thus, a new demand has been created recently for feasible and safe methods that can increase the levels of bioactive compounds in fresh horticultural produce (Ruiz-Garcia and Gomez-Plaza, 2013; Schreiner and Huyskens-Keil, 2006). Mango (*Mangifera indica* L.) is a fruit rich in several health-beneficial phytochemicals (Masibo and He, 2008; Masibo and He, 2009; Saleem, 2009, Kim et al., 2007; Papafox-Carlos et al., 2012a and 2012b) which is ranked fifth among the most cultivated and traded fruit crops in the world (FAOSTAT, 2017). Hence, improving the levels of important phytochemicals in mango fruit may cause a significant impact on the health prospects of the consumers. Further, it would benefit growers, traders and the food processing and nutraceutical industries around the globe by providing more opportunities to capitalize in health-conscious markets. Thus, different methods and conditions that can be employed to increase the concentrations of important phytochemicals - lupeol, mangiferin, phenolic acids such as gallic, chlorogenic, vanillic, ferulic, caffeic, and total phenols, antioxidants, carotenoids and ascorbic acid in the pulp and peel of mango fruit at eating soft stage were investigated in this thesis.

Based on the literature, application of Mg^{2+}, Mn^{2+} and Fe^{2+} as enzyme cofactors in terpenoid biosynthesis (Fischbach et al., 2000; Lucker et al., 2002; McGarvey and Croteau, 1995; Rohdich et al., 2000), the process of ripening (Ruiz-Montañez et al., 2014; Srivastava et al., 2015), low temperature stress (Ruiz-Garcia and Gomez-Plaza, 2013) and chemical elicitor treatments such as methyl jasmonate (MeJA), salicylic acid (SA) and nitric oxide (NO) (Ruiz-García, and Gómez-Plaza, 2013;

Schreiner and Huyskens-Keil, 2006; Zhao et al., 2005), were identified as possible determinants of the concentration of health-promoting compounds in the pulp and peel of ripe mango fruit. Cultivar 'Kensington Pride' (KP) was chosen for these experiments as it is the predominant mango cultivar grown in Australia (Horticulture Innovation Australia Limited, 2017). 'R2E2' is also a commercially important mango cultivar in Australia. Thus, R2E2 fruit were also used to determine the changes in the concentrations of health-promoting compounds during ripening to compare any varietal effect.

9.2 Discussion and conclusions

9.2.1 Pre-harvest application of mineral nutrients

The first objective of this study was to elucidate the effects of the pre-harvest spray application of aqueous solutions of mineral nutrients including $FeSO_4$ (0.2% and 0.3%), $MgSO_4$ (0.2% and 0.3%) and $MnSO_4$ (0.2% and 0.3%) one month before harvesting, on the concentrations of terpenoids (lupeol and total carotenoids) and different health-promoting compounds (mangiferin, phenolic acids, total carotenoids, ascorbic acid, total phenols and total antioxidants) in the pulp and peel of ripe 'Kensington Pride' mango fruit. Terpenoid biosynthesis is known to be induced by the addition of enzyme cofactors Fe^{2+}, Mg^{2+} and Mn^{2+} (Fischbach et al., 2000; Lucker et al., 2002; McGarvey and Croteau, 1995; Rohdich et al., 2000). Thus, it was hypothesized that the pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$, would increase the levels of terpenoids and other health-promoting compounds in ripe mango fruit. In agreement with the hypothesis, the concentration of triterpene lupeol in the peel was significantly higher with the pre-harvest spray application of aqueous solution containing 0.2% $FeSO_4$, $MgSO_4$ or $MnSO_4$. The level of total carotenoids (tetraterpenes) in the pulp of ripe mango fruit was significantly higher with the pre-harvest spray application of 0.3% of all above nutrients (Chapter 4, Fig. 4.2 and 4.3). Similarly, the concentrations of gallic, chlorogenic, ferulic and caffeic acids in the peel of ripe mango fruit were significantly highest with the pre-harvest spray application of 0.2% $FeSO_4$, whilst 0.2% of all nutrients could increase the level of mangiferin in the pulp compared to the untreated control (Chapter 4, Table 4.1 and

4.2). This study, for the first time, identified the pre-harvest spray application of $FeSO_4$, $MgSO_4$ and $MnSO_4$ at selected concentrations (0.2% and/or 0.3%) depending upon the health-promoting compound, as an effective tool to increase the levels of lupeol, total carotenoids, mangiferin and some phonolic acids in the pulp and/or peel of ripe mango fruit. The efficacy of pre-harvest application of these nutrients on improving levels of health-promoting compounds in ripe mango fruit in different commercial cultivars at multiple locations warrants to be investigated.

9.2.2 Harvest maturity

The second objective of this study was to examine the influence of different harvest maturities (standard green mature, sprung stage, half ripe and tree ripe) on the concentrations of lupeol, mangiferin, phenolic acids (gallic, chlorogenic, vanillic, ferulic, and caffeic), total phenols, total antioxidants, total carotenoids and ascorbic acid in the pulp and peel of 'Kensington Pride' mango fruit at eating soft ripe stage. Palafox-Carlos et al. (2012a) and Ruiz-Montanez et al. (2014) investigated the concentrations of health-promoting compounds in mango fruit just after harvesting at different maturity stages. According to them, 'Ataulfo' mango fruit harvested at riper stages from the tree had higher levels of lupeol, mangiferin (Ruiz-Montanez et al. 2014) and phenolic acids (Palafox-Carlos et al. 2012a). However, the influence of harvest maturity on the levels of the above mentioned bioactive compounds in ripe mango fruit harvested at different maturity stages is unknown. It was hypothesized that mango fruit harvested later than standard green mature stage may have higher levels of health-beneficial compounds. In agreement, the fruit harvested at the sprung stage resulted in the highest concentrations of lupeol, mangiferin, vanillic acid, ferulic acid and caffeic acid in both pulp and peel in ripe fruit and gallic acid, chlorogenic acid and total phenols in the peel, whilst the highest concentrations of ascorbic acid and total carotenoids in pulp and total antioxidant capacity in peel were recorded in the fruit harvested at tree ripe stage (Chapter 5, Section 5.3). The fruit harvested at half ripe stage had the highest antioxidant capacity in ripe pulp. Based on the results of the present study, for the first time, the sprung stage was identified as the best stage to harvest mango fruit over the standard green mature stage to obtain the maximum health benefits. However, the effects of different harvest

maturities on the cold and controlled atmosphere storage life and fruit quality are yet to be investigated.

9.2.3 Fruit ripening

Thirdly, the changes in concentrations of lupeol, mangiferin, phenolic acids (gallic, chlorogenic, vanillic, ferulic, caffeic), total phenols, total antioxidants, total carotenoids and ascorbic acid and the rates of ethylene production as well as respiration during mango fruit ripening were investigated. Previously, Srivastava et al. (2015) observed a significant compound, tissue and cultivar dependent influence of the process of ripening in the concentrations of lupeol and mangiferin in pulp and peel of different Indian mango cultivars. Thus in this thesis two independent experiments were carried out to explore the ripening related changes in the levels of health-beneficial bioactive compounds in 'Kensington Pride' and 'R2E2' mango fruit. In agreement with previous findings, a significant compound and tissue specific influence of the ripening process on the levels of health-promoting compounds was observed in the pulp and peel of the fruit of both cultivars especially after the climacteric ethylene and respiratory peaks recorded on day 3 (Chapter 6, Section 6.3; Vithana et al., 2017). A significant increase in the concentrations of lupeol, mangiferin, phenolic acids, total antioxidants and total carotenoids were observed in the pulp and/or peel of 'Kensington Pride' and 'R2E2' mango fruit during post-climacteric ripening phase (Chapter 6, Section 6.3; Vithana et al., 2017). Biosynthesis of secondary metabolites including polyphenols and terpenoids is induced during ripening as a pathogen defense (Ribeiro et al., 2008). Moreover, the production of phenolic antioxidants is triggered during this phase to shield the oxidative stress caused by the free radicals generated during respiratory climax (Masibo and He, 2008). In parallel with previous reports, the concentrations of all bioactive compounds were several-fold higher in the peel of mango fruit than pulp (Ruiz-Montañez et al., 2014; Srivastava et al., 2015). On the whole, mango fruit of major Australian cultivars 'Kensington Pride' and 'R2E2' at post-climacteric ripening phase was recognized as the best stage that could present the highest health benefits to its consumers, whilst the peel of these fruit could be used as a good source of these compounds for different industries such as food processing and

nutraceuticals after the extraction. This study for the first time demonstrated the changes in the concentrations of individual phenolic acids: gallic, chlorogenic, vanillic, ferulic and caffeic during postharvest ripening of mango fruit.

9.2.4 Chilling vs. standard low temperature storage

Next, the effect of chilling temperature (5 °C), over the standard non-chilling low temperature (13 °C) during storage for 12 d and 24 d prior to ripening at ambient temperature (21 ± 1.5 °C) on the concentrations of lupeol, mangiferin, phenolic acids (gallic, chlorogenic, vanillic, ferulic, caffeic), total phenols, total antioxidants, total carotenoids and ascorbic acid in the pulp and peel of ripe 'Kensington Pride' mango fruit was investigated. Chilling injury was also recorded at eating soft ripe stage. Physical elicitors such as low temperature stress are known to activate the biosynthesis of important secondary metabolites such as terpenoids and polyphenols by increasing the activity of phenylalanine ammonia lyase (PAL) (Cisneros-Zevallos, 2003; Ruiz-García and Gómez-Plaza, 2013). Thus, it was hypothesized that chilling temperature (5 °C) may increase the levels of health-promoting compounds in mango fruit compared to the standard low storage temperature (13 °C). In agreement, the concentrations of lupeol in pulp and peel and chlorogenic and caffeic acids in the pulp of ripe mango fruit were significantly higher after storage at 5 °C than 13 °C. Similar observations have earlier been reported by Rivera-Pastrana et al. (2010), in the concentrations of caffeic acid in 'Maradol' papaya fruit stored at 5 °C compared to 25 °C. However, mangiferin, gallic, chlorogenic, vanillic, ferulic, and caffeic acids, total phenols, antioxidants and carotenoids in the peel were significantly higher when stored at the standard cold storage temperature (13 °C). Significantly lower concentrations of lupeol and chlorogenic acid in pulp and peel and gallic acid in the pulp were observed when the fruit were stored for 24 d compared to 12 d, whilst an opposite trend was observed in the concentrations of vanillic acid, total phenols, total antioxidants and ascorbic acid in the pulp and caffeic acid in both pulp and peel (Chapter 7, Section 7.3). Thus, the influence of cold storage temperatures and duration on the concentration of health-promoting compounds in ripe mango fruit seems to be compound and tissue specific. Chilling injury symptoms were observed only in the ripe fruit previously stored at 5 °C for 24 d. The concentrations

of lupeol in pulp and peel, chlorogenic and caffeic acids in the pulp were significantly higher in fruit stored at 5 °C than 13 °C, whilst mangiferin, gallic, chlorogenic, vanillic, ferulic, and caffeic acids, total phenols, antioxidants and carotenoids in the peel were significantly higher when stored at 13 °C. The concentrations of lupeol and chlorogenic acid in pulp and peel and gallic acid in the pulp were significantly lower when stored for 24 d compared to 12 d, whilst vanillic acid, total phenols, total antioxidants and ascorbic acid in the pulp and caffeic acid in both pulp and peel were significantly higher when stored for 24 d. In conclusion, this study, for the first time demonstrated that storage of mature green mangoes at chilling temperature (5 °C) for 12 d prior to ripening (21 ± 1.5 °C) could significantly increase the level of lupeol in the pulp and peel of ripe mango fruit.

9.2.5 Post-harvest chemical elicitors

The final objective of this study was to investigate the effects of chemical elicitors; methyl jasmonate (MeJA), salicylic acid (SA) and nitric oxide (NO) on the concentrations of health-promoting compounds in ripe mango fruit. MeJA and SA are well-known chemical elicitors (Schreiner and Huyskens-Keil, 2006), whilst NO has more recently been identified as a plant signalling molecule (Zhao et al., 2005). The biosynthesis of secondary metabolites such as terpenoids, flavonoids, and other polyphenolic compounds is known to be induced by the cascade of signal transduction network initiates by an elicitor treatment (Cisneros-Zevallos, 2003; Halim et al., 2006; Rohwer and Erwin, 2008; van der Fits and Memelink, 2000; Zhao et al., 2005).Thus, three independent experiments were carried out to investigate the effects of the postharvest application of different concentrations of these three chemicals (10^{-4} M, 10^{-5} M and 10^{-6} M MeJA; 1.0 mmol L^{-1}, 2.0 mmol L^{-1} and 3.0 mmol L^{-1} SA and 10.0 µL L^{-1}, 20.0 µL L^{-1}, and 40.0 µL L^{-1}) at green mature stage of 'Kensington Pride' mango fruit on the concentrations of lupeol, mangiferin, phenolic acids (gallic, chlorogenic, vanillic, ferulic, caffeic), total phenols, total antioxidants, total carotenoids and ascorbic acid in the pulp and peel of ripe fruit. In agreement, the concentrations of mangiferin, gallic and chlorogenic acids, total phenols and antioxidants in both pulp and peel, total carotenoids and ascorbic acid in the pulp and lupeol and caffeic acid in the peel of ripe mango fruit were significantly increased

with MeJA fumigation for 24 h (10^{-5} M and/or 10^{-4} M MeJA) compared to untreated control. Dip treatment in SA solutions (2.0 mmol L^{-1} and/or 3.0 mmol L^{-1}) for 10 min could significantly increase the concentrations of chlorogenic and ferulic acids in both the pulp and peel and lupeol, mangiferin, vanillic acid, total carotenoids and ascorbic acid in the pulp of ripe mango fruit compared to the untreated control (Tables 8.1 and 8.2). However, the concentration of total phenol in the peel and gallic acid in both the pulp and peel were significantly lower with the SA treatment (Table 8.3 and 8.4). This could be a result of the suppression of respiratory climax in mango fruit by SA treatment (Srivastava and Dwivedi, 2000) as the biosynthesis of phenolic antioxidants could also be influenced by the accumulation of free radicals during respiratory climax (Masibo and He, 2008). The significantly lower concentrations of gallic acid may also have contributed to this reduction. The concentrations of lupeol, mangiferin, gallic and chlorogenic acids in the pulp and peel, vanillic, ferulic and caffeic acids, total phenols, total antioxidants, total carotenoids and ascorbic acid in the pulp of ripe mango fruit were significantly higher with the fumigation with NO for 2 h (20.0 and/or 40.0 μL L^{-1}). Overall, postharvest fumigation with 10^{-5} M and/or 10^{-4} M MeJA and 20.0 and/or 40.0 μL L^{-1} NO for 2 h and 2.0 mmol L^{-1} aqueous SA treatment for 10 min were identified, for the first time, as potential techniques to increase the levels of targeted phytochemicals in ripe mango fruit. More research work is needed to investigate the role of pre-harvest application of these chemical elicitors in commercially grown Australian mango cultivars.

9.3 Recommendations to the consumers and the industry

- Spray application of 0.2% aqueous $FeSO_4$ 30 d before harvest may be a viable commercial approach to obtain value added mango fruit with higher levels of lupeol, mangiferin, gallic, ferulic and caffeic acids in the peel and chlorogenic acid in the pulp for health-conscious consumers and related industries

- The standard harvest maturity (hard green mature) could be delayed up to sprung stage to obtain mango fruit with the highest concentrations of lupeol,

mangiferin, vanillic acid, ferulic acid and caffeic acid in both pulp and peel and gallic acid, chlorogenic acid and total phenols in the peel to cater for the health-oriented markets and related industries

- Health-conscious consumers, retailers, food additive and nutraceutical industries may obtain the maximum health benefits/ highest extraction of health-promoting compounds from the pulp and peel of mango fruit at post-climacteric ripening stage, which offers the highest levels of lupeol, mangiferin, phenolic acids, total antioxidants and total carotenoids

- Storage of mature green mango fruit at chilling temperature (5 °C) for 12 d before ripening at ambient temperature (21 ± 1.5 °C) seems to be a feasible commercial approach to obtain mangoes free from chilling injury and higher levels of lupeol in the pulp and peel of ripe fruit

- Fumigation with 10^{-5} M and/or 10^{-4} M MeJA for 24 h seems to be a practical method to increase the concentrations of mangiferin, gallic and chlorogenic acids, total phenols and antioxidants in both pulp and peel, total carotenoids and ascorbic acid in the pulp and lupeol and caffeic acid in the peel of ripe mango fruit to cater for the health-oriented markets and commercial industries

- Fumigation with 20.0 and/or 40.0 μL L^{-1} NO for 2 h is a safe and feasible method to increase the concentrations of lupeol, mangiferin, gallic and chlorogenic acids in the pulp and peel, vanillic, ferulic and caffeic acids, total phenols, total antioxidants, total carotenoids and ascorbic acid in the pulp of mango fruit

Disclaimer:

These recommendations are purely based on the laboratory and field experiments conducted under strictly controlled conditions. Discrepancies may arise in the outcomes under commercial situations. The project investigator Mekhala Dinushi

Kananke Vithana and Curtin University accept no liability whatsoever reason of negligence or otherwise arising from the reliance or use of these recommendations.

9.4 Future prospects

- Use of mass spectrometry in the determination of the concentrations of lupeol, mangiferin and phenolic acids and cellular assays for the determination of antioxidant potential would intensify the accuracy of the results in future studies.

- Investigating the dynamics in the activity of phenylalanine ammonia lyase (PAL) and the key enzymes involved in terpenoid and polyphenol biosynthetic pathways under different pre- and postharvest treatments and conditions may be helpful in better manipulation of these processes in future studies.

- Investigation of the role of chemical elicitors in the expression of gene(s) involved in terpenoid biosynthesis and shikimic acid pathway of polyphenol biosynthesis would explain the mechanism of action of these elicitors in enhancing the levels of different health promoting compounds in mango fruit.

- Clinical trials in humans to compare the ability of general and value added mango fruit in reducing the risk of selected degenerative diseases/s would be beneficial to decide new nutrient/dietary recommendations.

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